Resistance Screening to Fungal Diseases for Plantation Eucalypts in Vietnam; Molecular Tools to Assist Fungal Detection and Identification

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Declarations

I declare that this thesis does not contain any material which has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge, this thesis contains no material previously published or written by another person except where due acknowledgement is made.

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Abstract

Eucalyptus species are a significant component of the total forest plantation estate in Vietnam (23.6% in 2005). Fungal diseases have had a marked impact on eucalypt productivity and the area of plantation under *Eucalyptus* has halved over the last decade. Breeding programs have been implemented to address the problem of disease and have focused on the selection of species or clonal lines which have both high yield and disease resistance.

This thesis describes the resistance screening carried out in three clonal eucalypt trials planted in southern Vietnam. Sixty eucalypt clones and one seedlot (a landrace as a control) were assessed for growth traits, survival and crown damage. Approximately 50% of the clones, including the control, were classed as poorly performing clones based on the various criteria. Seventeen clones were significantly susceptible to disease at one or more sites. The limited number of clones ranked as top performers were those that were fast growing, with a high survival rate and resistance to disease. *Coniella* leaf spot was prevalent but was not systematically associated with high levels of damage. The more damaging pathogens present were *Cryptosporiopsis eucalypti*, *Cylindrocladium quinqueseptatum*, *Kirramyces destructans*. *Microsphaeropsis globulosa* on eucalypts was recorded for the first time on *Eucalyptus* in Vietnam. Although the major fungal pathogens are usually easily recognisable there are ambiguities in identification in the field. Future resistance screening and associated epidemiological studies will benefit from molecular techniques to detect and identify fungi, especially methods that can be applied directly to plant tissue.

Due to quarantine issues it was not possible to develop molecular tools to detect and identify fungal pathogens using infected tissue collected from the clonal eucalypt trials in Vietnam. Methodologies for subsequent use in Vietnam were developed using locally available material in Tasmania, within the context of an ongoing ecological investigation of the wood decay fungi associated with the logs of *Eucalyptus obliqua* in the wet sclerophyll forests of southern Tasmania.

Before attempting to identify fungi directly from fragments of decayed log, a reference library of ITS sequences was established from the DNA analysis of 111 sporocarps and 93 wood decay isolates obtained from a previous study (which had been grouped according to morphology). An additional 70 fungal cultures were obtained from the 111 sporocarps collected in the study. Sporocarps were morphologically identified to a genus or species level or had been given a tag name while awaiting formal identification. rDNA was amplified from DNA extracted from sporocarp tissue and fungal cultures. The internal transcribed spacer (ITS) region of the rDNA was sequenced. DNA was extracted from fragments of rotted wood from *E. obliqua* logs and the rDNA ITS amplified with fungal specific primers. The PCR products were cloned before sequencing as multiple fungal species were present in most samples. Clones were screened by PCR-RFLP and representatives of each PCR-RFLP group were sequenced.

To identify the fungi present in the rotted wood samples, sequences were compared to those from public and private databases, including the reference database set up within this study. The advantages and problems associated with identifying fungi directly from plant tissue using molecular techniques and the relevance of such methodology to resistance screening in Vietnam is discussed.

Acknowledgements

During the course of my study I was supported by a Vietnamese Government Postgraduate Award. Fieldwork in Vietnam was supported by the project "Screening for the selection of *Eucalyptus* and *Acacia* species for high productivity and disease resistance" led by Associate Professor Dr. N.H. Nghia, Director of the Forest Science Institute of Vietnam (FSIV). I am grateful to the School of Agricultural Science, University of Tasmania for providing conference funding to attend the 8th International Conference of Mycology, held in Cairns, Queensland, Australia in 2006. I also aknowledge ACIAR and the School of Agricultural Science, University of Tasmania for funding for my English course at the University of Tasmania.

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I gratefully thank my co-supervisors Dr. Morag Glen and Dr. Karina Potter, Ensis (a joint venture between CSIRO in Australia and SCION in New Zealand) in Hobart who tutored me in molecular biology and guided the molecular analyses of my fungi. They also contributed support in the thesis writing process.

I deeply acknowledge the statistical advice and help from my co-supervisor Dr. Chris Harwood, Ensis, Hobart.

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I would like to thank Dr. Anna Hopkins who also assisted with molecular work and provided sequences for the fungal isolates originating from the culture collection of wood decay fungi held at Ensis, Clayton in Australia. And also many thanks to the staff and students at the Ensis and the Cooperative Research Centre for Forestry who provided useful resources, discussion groups and friendship.

Lastly, I would like to thank Mr. N.V. Chien, Mr. T.H. Bien, South Eastern Forest Science and Product Centre, who provided me with information about the clonal eucalypt trials that I studied, Mr. D.T. Tan, my colleague at FSIV, who assisted with assessment and field work in Vietnam and also my colleagues at FSIV in Vietnam who encouraged me to do my Masters degree.

Table of taxonomic authorities for tree and fungal species[1]

Amauroderma rude (Berk.) Torrend
Amauroderma subresinosum (Murrill) Corner
(Current name = *Ganoderma subresinosum* (Murrill) Humphrey)
Anthracophyllum lateritium (Berk. & M.A. Curtis) Singer
Antrodia sinuosa (Fr.) P. Karst.
Antrodia xantha (Fr.) Ryvarden
Antrodiella zonata (Berk.) Ryvarden
Armillaria calvescens Bérubé & Dessur.
Armillaria fumosa Kile & Watling
Armillaria hinnulea Kile & Watling
Armillaria luteobubalina Watling & Kile
Armillaria novae-zelandiae (G. Stev.) Boesew.
Armillaria pallidula Kile & Watling
Aspergillus restrictus G. Sm.
Auriscalpium villipes (Lloyd) Snell & E.A. Dick
Beauveria tenella (Sacc.) Siemaszko
(Current name = *Beauveria brongniartii* (Sacc.) Petch)
Bisporella citrina (Batsch) Korf & S.E. Carp.
Boidinia propinqua (H.S. Jacks. & Dearden) Hjortstam & Ryvarden
Boletus edulis Bull.
Bridgeoporus nobilissimus (W.B. Cooke) T.J. Volk, Burds. & Ammirati
Cadophora malorum (Kidd & Beaumont) W. Gams
Calonectria quinqueseptata Figueiredo & Namek.
Candida fragi M. Suzuki, Nakase & Fukaz.
Cerrena unicolor (Bull.) Murrill
Collybia cirrhata Sensu Cooke
(Current name = *Collybia cookei* (Bres.) J.D. Arnold)
Coniella australiensis Petr.
Coniella fragariae (Oudem.) B. Sutton
Coniophora puteana (Schumach.) P. Karst.
Coniothyrium zuluense M. J. Wingf., Crous & Coutinho
Cortinarius alboviolaceus (Pers.) Fr.
Cryptosporiopsis eucalypti Sankaran & B. Sutton,
Cryphonectria cubensis (Bruner) Hodges
Cryphonectria gyrosa (Berk. & Broome) Sacc. & D. Sacc.
Cylindrocladium quinqueseptatum Boedijn & Reitsma
Dermocybe cardinalis E. Horak
Diplomitoporus lindbladii (Berk.) Gilb. & Ryvarden
(Current name = *Poria lindbladii* (Berk.) Cooke)
Echinodontium taxodii (Lentz & H.H. McKay) H.L. Gross

[1] Taxonomic authorities of the fungi and trees have been removed from the text to improve ease of reading

Table of taxonomic authorities for tree and fungal species (continued)

(Current name = *Laurilia taxodii* (Lentz & H.H. McKay) Pouzar)
Eichleriella deglubens (Berk. & Broome) Lloyd
Erythricium salmonicolor (Berk. & Broome) Burds.
(Current name = *Phanerochaete salmonicolor* (Berk. & Broome) Jülich)
Eucalyptus delegatensis R.T.Baker
Eucalyptus obliqua L'Hérit
Eucalyptus regnans F.Muell.
Fistulina hepatica (Schaeff.) With.
Fomes hemitephrus (Berk.) Cooke
Fomitiporia australiensis M. Fisch., J. Edwards, Cunningt. & Pascoe
Fomitopsis rosea (Alb. & Schwein.) P. Karst.
Galerina calyptrata P.D. Orton
Ganoderma applanatum (Pers.) Pat.
(Current name = *Ganoderma australe* (Fr.) Pat.)
Ganoderma fornicatum (Fr.) Pat.
Ganoderma philippii (Bres. & Henn. ex Sacc.) Bres.
Gloeophyllum sepiarium (Wulfen) P. Karst.
Gloeoporus taxicola (Pers.) Gilb. & Ryvarden
(Current name = *Meruliopsis taxicola* (Pers.) Bondartsev)
Grifola sordulenta (Mont.) Singer
Gymnopilus allantopus (Berk.) Pegler
Gymnopilus junonius (Fr.) P.D. Orton
Gymnopilus pampeanus (Speg.) Singer
(Current name = *Pseudogymnopilus pampeanus* (Speg.) Raithelh.)
Gymnopilus robustus Guzm.-Dáv.
Gymnopilus tyallus Grgur.
Hymenoscyphus ericae (D.J. Read) Korf & Kernan
(Current name = *Rhizoscyphus ericae* (D.J. Read) W.Y. Zhuang & Korf)
Hypholoma fasciculare (Fr.) P. Kumm.
Hypholoma sublateritium (Schaeff.) Quél.
Hypochnicium albostramineum (Bres.) Hallenb.
Hypocrea koningii Lieckf., Samuels & W. Gams
Hypocrea sulphurea (Schwein.) Sacc.
Hypoxylon diatrypeoides Rehm
Hypoxylon rubiginosum (Pers.) Fr.)
(Current name = *Hypoxylon rubiginosum var. rubiginosum* (Pers.) Fr.)
Kirramyces destructans[2] M.J. Wingf. & Crous.
Kirramyces epicoccoides (Cooke & Massee) J. Walker, B. Sutton & I. Pscoe
Laccocephalum mylittae (Cooke & Massee) Nüñez & Ryvarden

[2] A Andjic, V., Barber, P.A., Carnegie, A.J., Hardy, G.E.S., Wingfield, M J. and Burgess, T.I. (2007a). Phylogenetic reassessment supports accommodation of *Phaeophleospora* and *Colletogloeopsis* from eucalypts in *Kirramyces*. MycologicalResearch 111: 1184-1198. changed the name back from *Phaeophleospora destructans* to *Kirramyces destructans*

Table of taxonomic authorities for tree and fungal species (continued)

Lasiodiplodia theobromae (Pat.) Griffon & Maubl.
Lentinellus pulvinulus (Berk.) Pegler
Marchandiomyces aurantiacus (Lasch) Diederich & Etayo
Microsphaeropsis callista (H. Syd.) B. Sutton
Microsphaeropsis globulosa (Sousa da Câmara) B. Sutton
Mortierella verticillata Linnem.
Mycelium radicis-atrovirens Melin
Mycosphaerella marksii Carnegie & Keane
Oidiodendron myxotrichoides M. Calduch, Gené & Guarro
Oidiodendron pilicola Kobayasi
Oidiodendron rhodogenum Robak
Oidiodendron setiferum Essl.
Oligoporus rennyi (Berk. & Broome) Donk
(Current name = *Postia rennyi* (Berk. & Broome) Rajchenb.
Ophiostoma grandicarpum (Kowalski & Butin) Rulamort
(Current name = *Ceratocystis grandicarpa* Kowalski & Butin)
Penicillium citreonigrum Dierckx
Penicillium spinulosum Thom
Penicillium thomii Maire
Peniophora aurantiaca Peniophora erikssonii
Peniophora cinerea (Pers.) Cooke
Peniophora incarnata (Pers.) P. Karst.
Peniophora limitata (Chaillet ex Fr.) Cooke
Pestalotiopsis microspora (Speg.) G.C. Zhao & N. Li
Phanerochaete carnosa (Burt) Parmasto
Phanerochaete sordida (P. Karst.) J. Erikss. & Ryvarden
Phellinus robustus (P. Karst.) Bourdot & Galzin
Phellinus senex (Nees & Mont.) Imazeki
Phellinus wahlbergii (Fr.) D.A. Reid
Phialocephala dimorphospora W.B. Kendr.
Phlebia radiata Fr.
Phlebia rufa (Pers.) M.P. Christ.
Phlebia tremellosa (Schrad.) Nakasone & Burds.
(Current name = *Merulius tremellosus* Schrad.)
Phlebia uda (Fr.) Nakasone
(Current name = *Mycoacia uda* (Fr.) Donk)
Pholiota carbonaria (Batsch) Singer
(Current name = *Pholiota highlandensis* (Peck) A.H. Sm. & Hesler)
Pholiota highlandensis (Peck) A.H. Sm. & Hesler
Pholiota spumosa (Fr.) Singer
Pleurotopsis longinqua (Berk.) E. Horak
Polyporus mylittae Cooke & Massee

Table of axonomic authorities for tree and fungal species (continued)

(Current name = *Laccocephalum mylittae* (Cooke & Massee) Núñez & Ryvarden)
Poria lindbladii (Berk.) Cooke
Postia balsamea (Peck) Jülich
Postia caesia (Schrad.) P. Karst.
Postia sericeomollis (Romell) Jülich
Postia subcaesia (A. David) Jülich
(Current name = *Tyromyces subcaesius* A. David)
Psathyrella echinospora
Psathyrella spadiceogrisea (Schaeff.) Maire
Pseudocercospora eucalyptorum Crous, M. J. Wingf. & Marasas
Psilocybe coprophila (Bull.) P. Kumm.
Pulvinula archeri (Berk.) Rifai
Pycnoporus cinnabarinus (Jacq.) Fr.
Pycnoporus coccineus (Fr.) Bondartsev & Singer
Rhodocollybia butyracea (Bull.) Lennox
Schizophyllum commune Fr.
Scytinostroma ochroleucum (Bres. & Torrend) Donk
Simplicillium lamellicola (F.E.V. Sm.) Zare & W. Gams
Sistotrema brinkmannii (Bres.) J. Erikss.
Skeletocutis amorpha (Fr.) Kotl. & Pouzar
Spongipellis spumeus (Sowerby) Pat.
Sporendocladia foliicola (P.M. Kirk) M.J. Wingf.
Steccherinum litschaueri (Bourdot & Galzin) J. Erikss.
Stephanoascus ciferrii M.T. Sm., Van der Walt & Johannsen
Stereum annosum Berk. & Broome
(Current name = *Xylobolus annosus* (Berk. & Broome) Boidin)
Stereum ostrea (Blume & T. Nees) Fr.
Stereum sanguinolentum (Alb. & Schwein.) Fr.
Thanatephorus cucumeris (A.B. Frank) Donk
Togninia minima (Tul. & C. Tul.) Berl.
(Current name = *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai)
Trametes hirsuta (Wulfen) Pilát
Trametes ochracea (Pers.) Gilb. & Ryvarden
Trametes versicolor (L.) Lloyd
Trichoderma album Preuss
(Current name = *Hypocrea citrina* (Pers.) Fr.)
Tyromyces chioneus (Fr.) P. Karst.
Tyromyces subcaesius A. David
Umbelopsis isabellina (Oudem.) W. Gams
Umbelopsis ramanniana (A. Møller) W. Gams
Xerula australis (Dörfelt) R.H. Petersen

Table of taxonomic authorities for tree and fungal species (continued)

Xerula furfuracea (Peck) Redhead, Ginns & Shoemaker
Xerula radicata (Relhan) Dörfelt
Xylaria castorea Berk.

Table of Contents

The letter A after Fig. is used in the text to refer to different figures in an Appendix e.g. Fig.A 2.2.1

Section 1: Assessment of clonal eucalypt trials in Vietnam for disease resistance

1. Introduction

Due to their economic, environmental and social significance, eucalypts are widely grown throughout Vietnam. *Eucalyptus* is one of the main tree species grown in the reforestation program in Vietnam (Kha 1996). Eucalypts were first introduced into Vietnam in the 1930s (Thiep 1996). During the period from 1986 to 2001, nearly half a million ha of eucalypts were planted in Vietnam and accounted for 46.5% of the total forest plantation area (MARD 2002). In 2005, eucalypt plantations made up approximately 348,000 ha out of the 1,471,394 ha of the total forest plantation area in Vietnam (FIPI 2005). By the year 2010, the Vietnamese Government plans to establish an additional five million ha of forest. Two million ha will be restoration of poor secondary-natural forest and three million ha will be newly planted areas. Two thirds of the newly planted areas will be *Acacia* and *Eucalyptus* plantations (Kha 1998).

Eucalypt plantations e.g. *Eucalyptus camaldulensis* (especially the Petford provenance) have a history of severe fungal diseases dating from the 1980s (Nghia 1992; Mao 1994; Sharma 1994; Old and Yuan 1995). In 1999 approximately half of 23,000 ha of eucalypt plantations in the four south-east provinces in Vietnam were reported to be significantly damaged by fungal diseases (Old 1999). It was also reported in 1999 from the Thua Thien Hue province of central Vietnam, that nearly all of the 7000 ha of eucalypt plantations in this region were infected by a wide suite of fungal leaf spots or blights e.g. *Cylindrocladium quinqueseptatum, Cryptosporiopsis eucalypti, Mycosphaerella marksii* (Banh 1999; Nghia 2006). Several authors have described the fungal leaf spots or blights prevalent on eucalypts in Vietnam; *Cylindrocladium quinqueseptatum* causing Cylindrocladium leaf blight (Peerally 1974; Pitkethley 1976; Sharma and Mohanan 1982; Sharma *et al.* 1985; Crous and Wingfield 1994), *Cryptosporiopis eucalypti* (Old et al. 2002, Sankaran *et al.* 1995), *Coniella* spp. (Sutton 1980), *Pseudocercospora* spp. or their *Mycosphaerella* sexual states (Crous 1998). Species of *Cylindrocladium* has also been recorded as fungal pathogens in countries such as in Australia (Bolland *et al.* 1985; Cromer *et al.* 1991), in South Africa (Booth *et al.* 1989; Crous *et al.* 1993) and in India (Sharma and Mohanan 1982; Sharma *et al.* 1985).

Disease incidence and severity was probably exacerbated as a result of the widespread cultivation of highly susceptible eucalypt provenances in climates associated with high disease risk (Booth *et al.* 2000). There was evidence to show that it should be possible to select for disease resistance in eucalypts in Vietnam (Jayasree *et al.* 1986; Sharma and Mohanan 1992). A project "Minimizing disease impacts on eucalypts in south-east Asia" was undertaken between 1996 and 2000 (Old 2001) to reduce the damage caused by fungal leaf spots and blights to eucalypts grown in sub-tropical Australia, Vietnam and Thailand. In this project, Nguyen (1999) assessed the growth and disease in an *E. camaldulensis* provenance/progeny trial in south-east Vietnam. Nguyen's results showed that the most significant diseases on eucalypts were *C. eucalypti*, *C. quinqueseptatum*, and a species of *Pseudocercospora*. His results also showed some of the best performing eucalypt provenances, in terms of growth and disease resistance, were Katherine, Morehead River and Kennedy River. The Petford eucalypt provenance performed poorly. There was a clear negative correlation between height and disease index (R=0.6 to 0.9).

Several trials were established in 2003 in different environments in Vietnam to screen for both yield and disease resistance planted as another phase of Vietnam's eucalypt selection program. These trials include widely tested second and third generation selection of eucalypt germplasm (selections from the trial described in Nguyen 1999; Nghia 1999, 2004) and other less well known clones (in an attempt to broaden the genetic base of clones in Vietnam). The aim of this section of this thesis was to carry out an initial assessment of the growth and disease resistance in these eucalypt clonal trials.

2. Materials and methods

2.1. Site descriptions

Location of trial sites (Fig.2.1.1 and Table 2.4.1) and time of planting: The trials in Song May and Bau Bang were planted in August, 2002; the trial in Minh Duc was planted in August, 2003. These three sites were selected as they were the most representative of the different types of forest plantation found in southern Vietnam in respect to terrain, humidity, soil type etc. These sites were also known to be in locations favourable to the development of fungal disease epidemics such as leaf blight (Old *et al.* 2000a; Old 2001; Nghia 2004).

At the Minh Duc and Song May sites the relative humidity is 70 - 80%. The sites are flat with a deep grey-fluvisol soil of low nutrient status and underlain by a heavy clay pan. This soil dries out rapidly and is easily flooded. At Bau Bang the relative humidity is also 70 - 80%, the site slopes gently (8-15^{o}) and the soil type is a typical grey-fluvisol but shallow.

For more information about the soil physical and chemical properties at all three sites, see Table 2.1.1.

The mean annual rainfall at all three sites is very similar and in the range 1800-2200 mm. The rainy season is from May to October and the dry season is from November to April.

Figure 2.1.1: Map of site locations (in red text) for eucalypt clonal trials in Vietnam.

Trial	Latitude (^{o}N)	Longitude (^{o}E)	Altitude (m)
Minh Duc	$11^{0}36$	$106^{0}32$	10
Song May	$11^{0}03$	$106^{0}58$	50
Bau Bang	$11^{0}15$	$106^{0}38$	10

Table 2.1.1: Soil physical and chemical properties at the trial sites.

No.	Factor	Soil depth (cm)	Site		
			Minh Duc	Song May	Bau Bang
1	pH KCl	0-20	4.29	4.34	4.31
		20-40	4.34	4.24	4.45
2	Humus (%)	0-20	2.00	1.20	1.42
		20-40	1.16	0.88	0.89
3	Nitrogen (%)	0-20	0.100	0.049	0.057
		20-40	0.088	0.025	0.028
4	C/N ratio	0-20	11.6	14.0	14.3
		20-40	11.6	20.0	18.5
5	P_2O_2 (mg/100g)	0-20	1.79	2.31	3.68
		20-40	0.74	1.10	2.99
	K_2O	0-20	4.22	1.81	3.01
		20-40	2.41	2.41	1.20
6	Hydrolysis (mg/100g)	0-20	4.13	3 49	3.96
		20-40	3.65	4 29	3.69
7	Exchangeable Al^{+++} (mg/100g)	0-20	0.82	0 82	0.91
		20-40	1.00	1.54	0.99
	Exchangeable H^+ (mg/100g)	0-20	0	0	0
		20-40	0	0	0
8	Exchangeable Ca^{++} (mg/100g)	0-20	0.81	1.00	0.51
		20-40	0.41	0.50	0 31
	Exchangeable Mg^{++} (mg/100g)	0-20	0.50	0.71	0.30
		20-40	0.10	0.41	0.20
9	Soil texture 2-0.02	0-20	73.79	73.82	85.90
		20-40	35.55	67.71	79.86
	Soil texture 0.02 -0.002	0-20	8.06	8.06	8.06
		20-40	44.31	6.05	8.06
	Soil texture < 0.002	0-20	18.14	18.13	6.04
		20-40	20.14	26.23	12.08

2.2. Plant material

A total of 60 eucalypt clones were planted at three trial sites, but not all clones were tested at each site. 34 clones were each tested at a single site, 21 clones were each tested at 2 sites and 5 clones were each tested at 3 sites (Appendix 1.1). At each site, there was a control treatment using seedlings originating from a landrace routinely used for plantings of *E. camandulensis* in Vietnam. The 60 eucalypt clones were derived from different locations in Vietnam and two from China (see Appendix 1.1 for origin of clones).

Two clones (9905 and 9906) were imported from China. They were were selected for this trial as they were considered as advanced varieties by the Ministry of Agricultural and Rural Development of Vietnam (Nghia 2003, 2006).

Two clones (9910 and 9911) were selections by the South Eastern Forest Science and Product Centre, originated from a previous eucalypt trial at Bau Bang (Fig.2.1.1).

Seven clones (9107, 9410, 9495, 9498, 9501, 9518, and 9904) had been assessed as the best clones in a trial planted in 1998 at Song May (Fig.2.1.1).

Fourteen clones (350, 501, 1902, 2202, 3302, 3502, 3602, 3702, 4202, 4902, 7301, 9901, 9903, 9908) were selected from the provenance-progeny trial of 150 eucalypt families planted in 1996 at Chon Thanh (Fig.2.1.1) (Nguyen 1999).

Two clones, 9907 and 9912, (hybrids between *E. camaldulensis x urophylla*) came from vigorous trees assessed as good-performing clones and had been selected by the Phu Linh Paper Research Centre, in northern Vietnam (Phu Tho province, see Fig.2.1.1).

The remaining thirty-three clones were selected from the healthiest individuals in the midst of epidemics from other trials and production forest plantations throughout Vietnam (see Nghia 2003).

2.3. Site preparation

Before planting existing vegetation was cleared and the sites were disc ploughed. Weed control was carried out in the June and December of each planting year. A tractor ploughed in weeds between planting rows and weeds were manually removed along rows. NPK fertilizer obtained from Song Gianh Fertilizer Ltd. company was applied at planting, 150 g/tree, equivalent to 250 kg/ha.

There are considerable areas of eucalypt plantations and numbers of scattered eucalypt trees close to all trials. Neighbouring plantings of *E. camaldulensis* had been experiencing fungal disease problems over a period of several years prior to planting the trial described in this thesis (Nghia 2004). CSIRO forest pathologists had identified the following pathogens and associated fungal diseases (Old 2001):

+ Cryptosporiopsis leaf spot and shoot die back – *Cryptosporiopsis eucalypti* (Figures A1.4.4 to A1.4.6a).

+ Coniella leaf spot – *Coniella fragariae* (Figures A1.4.1 and A1.4.3a).

+ Cylindrocladium leaf blight - *Cylindrocladium quinqueseptatum* (Fig.A1.4.9).

Plantation trees or coppice was retained next to trial sites to act as disease inoculum sources.

2.4. Trial design

Randomized complete block designs were applied at all three sites but plot size and number of replicates varied from site to site (Table 2.4.1).

Table 2.4.1: Experimental designs for the 3 *Eucalyptus* clonal trials.

Trial site	Province	No. of treatments[1]	Plot size	No. of replicates
Minh Duc	Binh Phuoc	44	4 trees	8
Song May	Dong Nai	40	5 trees	5
Bau Bang	Binh Duong	10	20 trees	4

[1]: Treatments included one control planting of *E. camaldulensis*

At the Minh Duc trial site: there were 8 replicates, each containing 2 incomplete blocks in column (col-block) and 22 incomplete blocks in row (row-block). Each col-block contained 22 plots; each row-block contained 2 line plots. Each line plot consisted of four trees of one clone. Spacing was 3 m x 1.5 m.

At the Song May trial site: there were 5 replicates, each containing 4 incomplete blocks in column (col-block) and 10 incomplete blocks in row (row-block). Each col-block contained 10 plots; each row-block contained 4 line plots. Each line plot consisted of four trees of one clone. Spacing was 3 m x 1.5 m.

At the Bau Bang trial site: there were 4 replicates, each containing 10 complete blocks in column. Each column consisted of twenty trees of one clone. Spacing was 3 m x 1.5 m.

2.5. Assessments

Assessments were carried out in September, 2004; the Song May and Bau Bang trials were therefore assessed 29 months after planting, and the trial at Minh Duc was assessed 17 months after planting.

Height was measured with height poles to the nearest 0.5 m; stem diameter over bark at breast height (dbh) was measured using diameter tapes to the nearest 0.5 cm at Song May and Bau Bang sites, and diameter at ground level (dgl) was measured at Minh Duc trial.

Crown damage index (score): Disease assessment methods described by Old (2001) were applied to score crown damage in individual trees. An individual tree could be allocated a score of 1 through to 6 in increments of 1. Code 1 equated to a nil or low level of premature crown defoliation or chlorosis resulting from fungal infection whereas code 6 represented total or almost total defoliation and dieback. The intermediate codes 2-5 increased stepwise in severity relative to percent of crown damaged by fungal disease. Only the main diseases present on each tree were scored. Where a disease occurred on only a very small number of leaves it was not recorded. Six tree photographs representing codes 1 to 6 are given in Appendix 1.2.

The following codes for individual disease were used when scoring for crown damage index (Old 2001).

A: *Cryptosporiopsis eucalypti* (Figures A1.4.4 to A1.4.6a).

B: *Coniella fragariae* (Fig.A1.4.1).

C: *Cylindrocladium quinqueseptatum* (Fig.A1.4.9).

D: *Kirramyces destructans* (red/purple blotch symptom) (Figures A1.4.12 and A1.4.13).

E: *Pseudocercospora "eucalyptorum"* (sooty lesion with associated chlorosis/ red margin).

F: *Pseudocercospora "eucalyptorum"* variant (sooty brown lesion without chlorosis).

G: Small prolific corky leaf spots ("a physiological response symptom" but commonly associated with *Cryptosporiopsis eucalypti*).

H: General chlorosis/ or chlorotic flecking of leaves.

I: Insect damage such as leaf and shoot browsing (Fig.A1.4.15).

J: Shoot dieback or blight sometimes of uncertain cause but commonly associated with *Cryptosporiopsis eucalypti.*

K: Black/ brown mildew (*Meliola* sp.), spotting commonly associated with the mid rib of a leaf.

U: Unknown cause.

Fungal pathogens observed in the trials were morphologically identified to genus or species levels with the assistance of morphological taxonomists.

2.6. Statistical analysis

Analysis of variance was carried out on plot mean values using the ANOVA directive in Genstat 7.1 software package (Genstat 2003). For the analysis of data from individual sites, replicates and clones were treated in the model as fixed effects (William *et al.* 2002). For the combined site analysis, clone means from each site were analysed using the FIT directive in Genstat 7.1 to examine clone-by-site interactions and the significance of these interactions was tested using a pooled residual mean square calculated from the individual analyses as described by Williams *et al.* (2002). Because stem diameters were measured at different heights at the different trial sites, only three variables (height, survival and crown damage index) were compared in the combined analysis.

3. Results

3.1. Minh Duc site (44 clones)

Height: Clone mean heights ranged from 2.9 to 5.6 m (Fig.3.1.1). Average mean height across the trial was 4.5 m. There were three clones (2802, 3702 and 4202) with heights equal to average mean point (4.5 m). There were eighteen clones with heights less than the mean point for the trial, accounting for 40.9% of all clones. Of these 18 clones, heights for six clones (201, 401, 601, 701, 5402 and 9907) were significantly less (p=0.001) than the mean height. These six clones probably represent the poorest performing clones at this site. Although the heights of these six clones were not significantly different from those of the other 12 clones with heights below the mean point they were significantly smaller than those clones with heights above the mean point.

The heights for the landrace control and twenty-two clones fell above mean point, accounting for 51% of all clones. Eight of these 23 clones (9518, 9902, 9903, 1501, 9498, 7301, 3402, and 9905) were ranked from 1 to 8 (Appendix 1.3.1) and were significantly taller (p=0.001) than clones below the mean point although not significantly different to the other 15 clones with heights above the mean point. They were considered the best performing clones at this site. On average, the height of these superior clones was 5.3 m (equivalent to a growth rate of 31.2 cm per month).

Diameter at ground level: Clone mean diameters ranged from 2.1 to 4.7 cm (Fig.3.1.2). The average mean clone diameter was 3.7 cm. There were four clones (302, 1302, 6402 and 9107) with diameters equal to this mean point of 3.7 cm. There were twenty-one clones with diameters below the mean point, accounting for 47.7% of all clones; six of these 21 clones (201, 401, 601, 701, 4802 and 5402) were significantly smaller (p=0.001) than clones with diameters above the mean point.

The landrace control and eighteen clones had diameters above the mean point, accounting for 45.4% of all clones; eight of these 19 clones (9903, 9905, 9518, 9902, 4402, 9495, 1001, and 1501) had significantly larger diameters (p=0.001) to clones below the mean point and were ranked as the highest performers (Appendix 1.3.1). The mean diameter for these eight clones was 4.5 cm.

Survival: Survival rates ranged from 31.2 to 90.6% (Fig.3.1.3). On average, clone survival rate was 69.2%. There were sixteen clones with survival rates less than the mean point, accounting for 36.4% of the total number of clones tested at this site; the survival rates for seven of these 16 clones (402, 601, 701, 2002, 4602, 5402 and 9907) were significantly lower (p=0.001) than those of other clones falling above the mean point.

The survival rates for the landrace control and twenty-seven clones (66%) were higher than mean point, and there were five clones (9903, 4402, 3802, 9518 and 2702) which were significantly survival higher (p=0.001) than the other clones, they were also at the top of the highest survival rate clones at the site.

Crown damage index: Scores at Minh Duc ranged from class 1 to class 5, there was no class 6. Mean clone crown damage indices (scores) ranged from 1.1 to 5.3 (Fig.3.1.4). Most of the clones at this site had a low level of disease and a total of twenty-four clones, including the control landrace, had crown damage indices below the mean for the site (2.5) accounting for 54.5% of the total number of clones. Clones with crown damage scores in classes 1 and 2 represented, respectively, 38.6% and 36.4% (= 75%) of the total number of clones at this site (Fig.3.1.5). A total of 19 clones were considered the best performing clones in respect to crown damage indices at this site; six clones (1001, 1501, 3402, 9518, 9903 and 9904) with significantly lower (p=0.001) scores than all other clones at the site (Fig.3.1.4) and the thirteen clones (101, 2002, 2702, 3702, 4202, 4402, 4502, 5102, 5402, 7301, 9410, 9495 and 9902) with scores under 2.

Clones with crown damage indices in classes 3 and 4 represented, respectively, 9.1% and 13.6% of the total number of clones at this site, one clone scored class 5 (Fig.3.1.5). In total sixteen clones were ranked higher than the mean crown damage index (2.5), representing 36.4% of the total number of clones at the site. But only eight of these clones (902, 4802, 401, 5202, 9908, 9907, 701 and 6402) had significantly higher crown damage indices (p=0.001) than other clones (Fig.3.1.4), representing 18.2% of the total number of clones at the site.

Fungal pathogens (Appendix 1.4): *Coniella* leaf spot was present on ten clones scored as class 1. Only one clone scored as class 1 was infected by *Cryptosporiopsis eucalypti*.

Coniella leaf spot was the main disease observed in eight clones scored as class 2. *C. eucalypti* was only observed on one clone scored as class 2.

Coniella leaf spot was also responsible for most of the crown damage in clones with crown damage index of 3 and 4 (two clones in class 3 and three clones in class 4). *C. eucalypti* was associated with damage to one clone in class 4 (Fig. 3.1.6).

The fungal agents causing damage to 20 clones could not be identified.

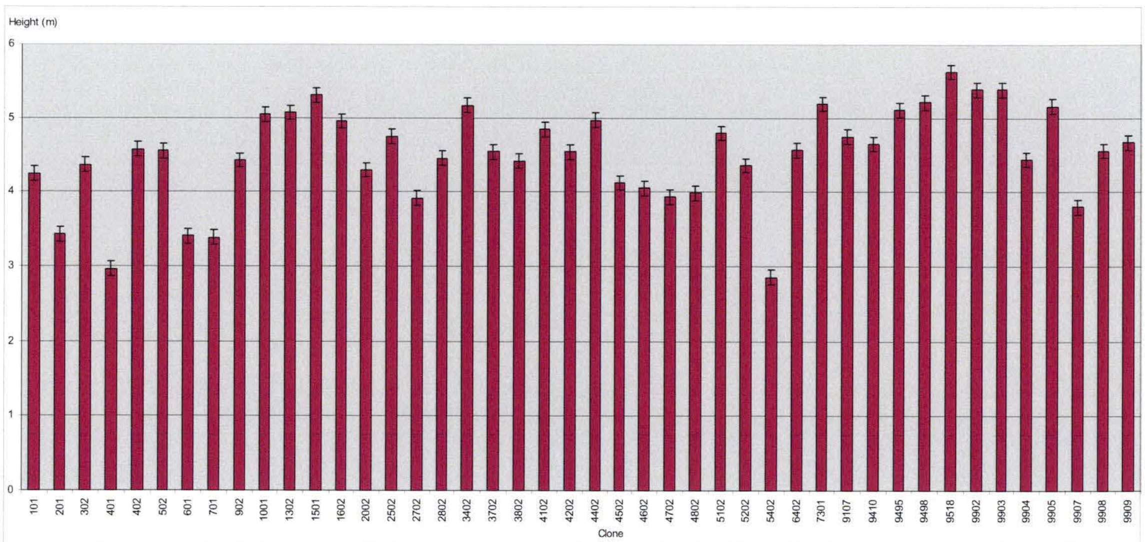


Figure 3.1.1: The average heights of clones and landrace (9909) at Minh Duc trial. The bar denotes standard error.

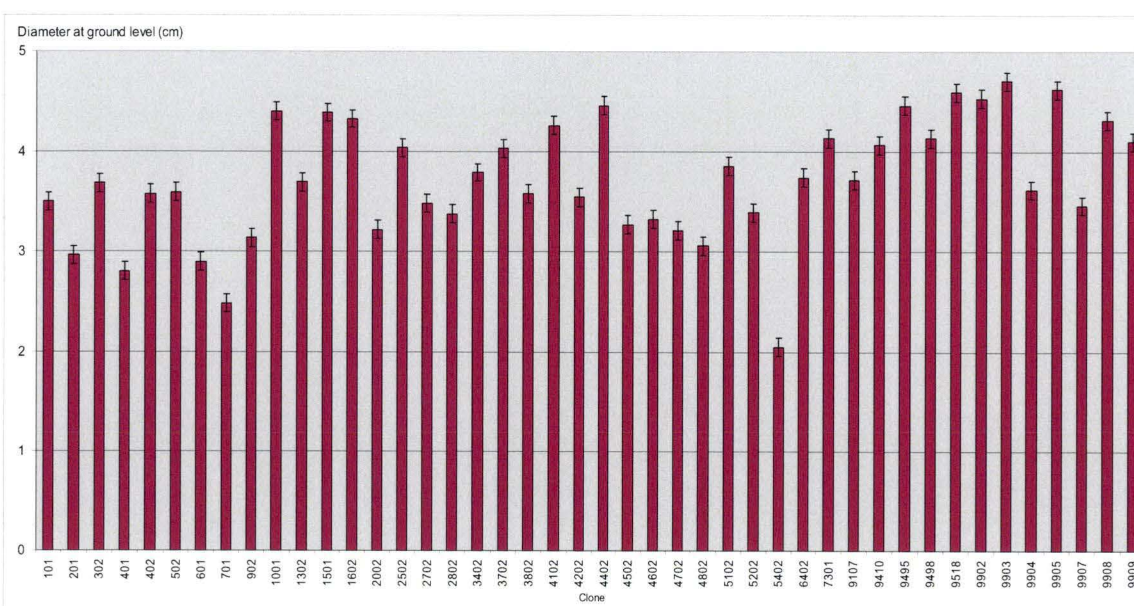


Figure 3.1.2: The average diameters at ground level of clones and landrace (9909) at Minh Duc trial. The bar denotes standard error.

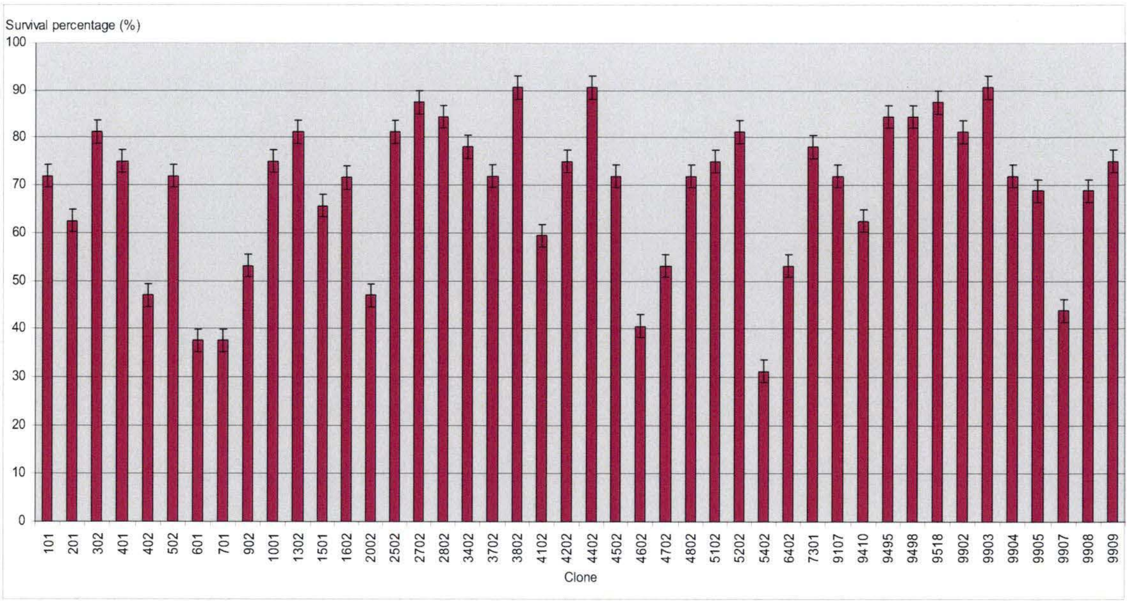


Figure 3.1.3: The average survival rates of clones and landrace (9909) at Minh Duc trial. The bar denotes standard error.

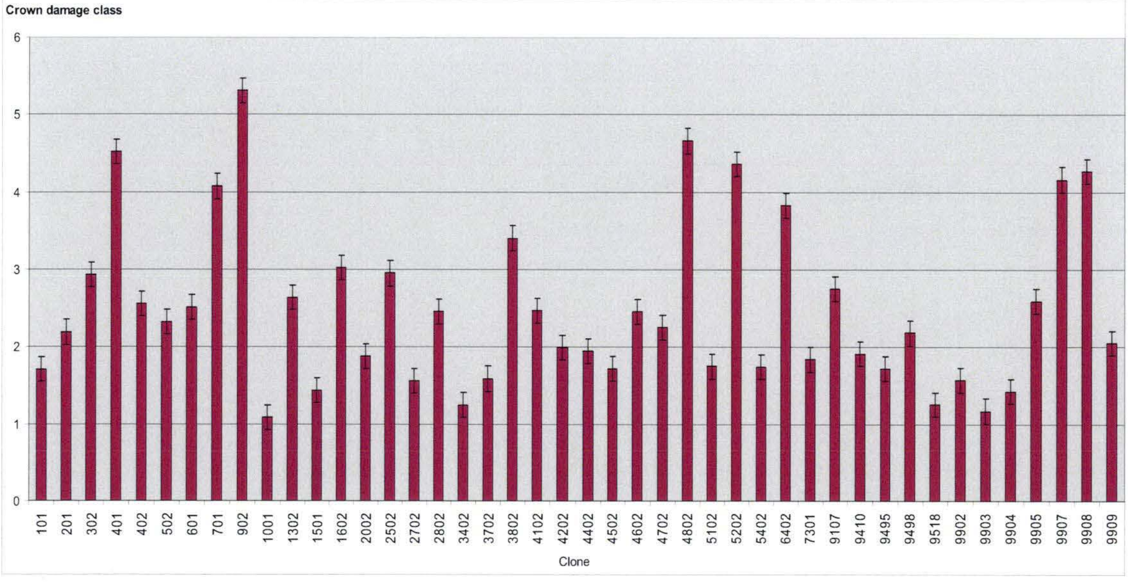


Figure 3.1.4: The average crown damage indices of clones and landrace (9909) at Minh Duc trial. The bar denotes standard error.

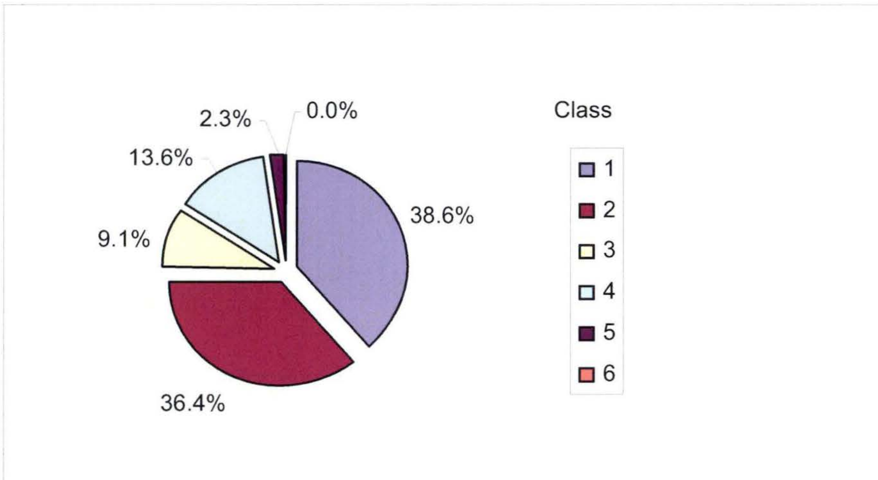


Figure 3.1.5: Crown damage index at Minh Duc site; class 1 is a healthy crown, class 6 is a severely damaged crown, classes 2-5 increase stepwise in the severity of crown damage.

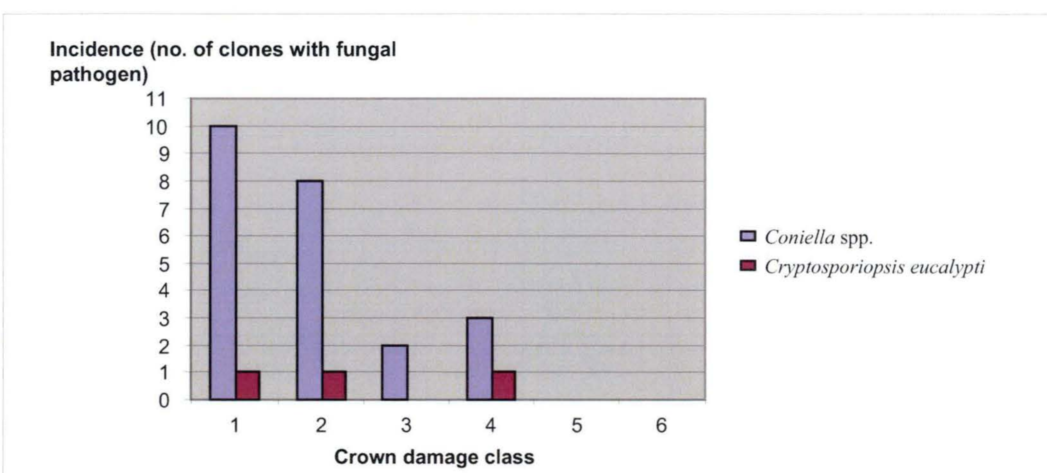


Figure 3.1.6: Incidence of fungal pathogens in each of 6 severity classes at Minh Duc.

3.2. Song May site (40 clones)

Height: Clone mean heights ranged from 5.7 to 8.8 m (Fig.3.2.1). On average, mean height was 7.1 m. There were twenty clones (i.e. 50% of the clones tested at this site) including the landrace control with mean heights below the mean point for all clones. The mean heights for seven of these 20 clones (201, 601, 2202, 4802, 5302, 5402 and 9501) were significantly lower (p=0.001) than the height mean point and therefore represent the poorest performers at this site in respect to height.

Mean heights for nineteen clones (47.5% of the total number of clones on site) fell above the mean height point. Seven of these 19 clones were significantly higher (p=0.001) than clones below the mean point and were ranked from 1 to 7 as the best performing clones on site in respect to height (i.e. 9495, 9518, 3502, 1501, 402, 9498 and 1602, see Appendix 1.3.1). The average mean height for these best clones was 8.3 m with a growth rate of 28.6 cm per month.

Diameter at breast height: Clone mean diameters ranged from 4.8 to 8.6 cm (Fig.3.2.2). On average, clone mean diameter was 6.6 cm; three clones (350, 3602, and 9901) had mean heights equal to the mean point for all clones. There were twenty clones (i.e. 50% of the total number of clones on site) with mean diameters below the mean point. Seven of these 20 clones (201, 601, 4902, 5302, 5402, 9501, 9904) and 9909 (the landrace), were significantly smaller in diameter (p=0.001) than the other clones which fell above the diameter mean point.

There were seventeen clones with diameters above the mean point representing 42.5% of the total number of clones on site. Ten of these 17 clones had significantly greater diameters (p=0.001) than clones below the mean point. These ten clones (9495, 9518, 3502, 9905, 9498, 9107, 1602, 1501, 402, and 1001) were ranked as the 10 best performing clones in respect to diameter (Appendix 1.3.1). The average mean diameter of these 10 clones was 7.9 cm.

Survival: Survival rates were between 24 and 100% (Fig.3.2.3). On average, clone survival rate was 76.3%. There were eighteen clones with survival rates lower than the mean point for survival rate, accounting for 45% of the clones planted on site.

Out of these 18 clones, four (201, 5402, 9501 and 9910) and the control landrace (9909) had significantly (p=0.001) lower mean survival rates than the other clones with survival rates above the mean point for clone survival rate.

There were twenty-two clones with mean survival rates above the mean point for survival rate (55%). The survival rates of five clones (9107, 9905, 502, 1001 and 9495) were significantly higher (p=0.001) than the other 35 clones, and they were the top five highest survival rate clones. Their survival rates ranged from 96 to 100%.

Crown damage index: Crown damage indices at this site ranged from class 1 to class 5 (mean clone crown damage indices ranged from 1.4 to 5.8 (Fig.3.2.4) and the mean crown damage index was 3.0).

No clone was scored in class 6 and there were only two clones (i.e. 5% of the clones on site) that were scored as class 5. Clones scored as class 3 or 4 represented 28% and 15%, respectively, of the clones on site (Fig.3.2.5). Nineteen clones in total ranked above the mean point (3.0) accounting for 47.5% of all clones tested on site. Of these 19 clones, eight clones had crown damage scores significantly higher (p=0.001) than the other twenty-two clones falling above or equal to the mean point. These eight clones were 302, 5402, 3402, 501, 1002, 4802, 9910 and 9905 (i.e. 20% of all clones on site) and considered the most severely damaged clones at this site.

Approximately half of the clones at this site were given low crown damage indices and clones with classes 1 and 2 accounted for 13 and 39%, respectively, of total number of clones on site (Fig.3.2.5). There were twenty-one clones in total, including the control landrace, with crown damage scores lower than 3.0, representing 52.5% of all clones on site. Of these 21 clones, there were five clones (9495, 9410, 9518, 3502, 350) and the control landrace with significantly lower (p=0.001) scores than other clones with scores above the mean point (Fig.3.2.4). These five clones and control landrace were considered the best performing clones in respect to disease resistance at this site.

Fungal pathogens (Appendix 1.4): *Coniella* leaf spot was found on four clones in class 1 (9495, 9518, 9410 and 3520) and five clones in class 2. It was associated with moderately severe damage in four clones with crown damage indices of class 3 (5302, 3802, 9501 and 1902) and was the only pathogen present in the latter 3 clones.

Pestalotiopsis spp. were observed in six clones and associated with variable crown damage indices ranging from 1 to 4: clones 9410 and 3520 in class 1; clones 9901 and 602 in class 2; clone 5302 in class 3 and in combination with *Coniella fragariae*; clone 501 in class 4. Clone 5302 was also attacked by a new gall forming pest, *Leptocybe invasa* Fisher and La Selle (Mendel *et al.* 2004) (Fig.A1.4.15).

Species of *C. eucalypti* were observed in four clones but only associated with low levels of damage (clones 3502 and 9518 in class 1 and clones 1602 and 4602 in class 2).

Kirramyces destructans caused a low level (class 1) of damage to control landrace (9909) and a much greater degree of damage (class 4) to another clone (302).

Cylindrocladium quinqueseptatum was observed in three clones (clone 5002 in class 2; clones 5402 and 4802 in class 4).

Microsphaeropsis globulosa (Fig.A1.4.14) was observed on one clone (5102) with a crown damage score of class 3.

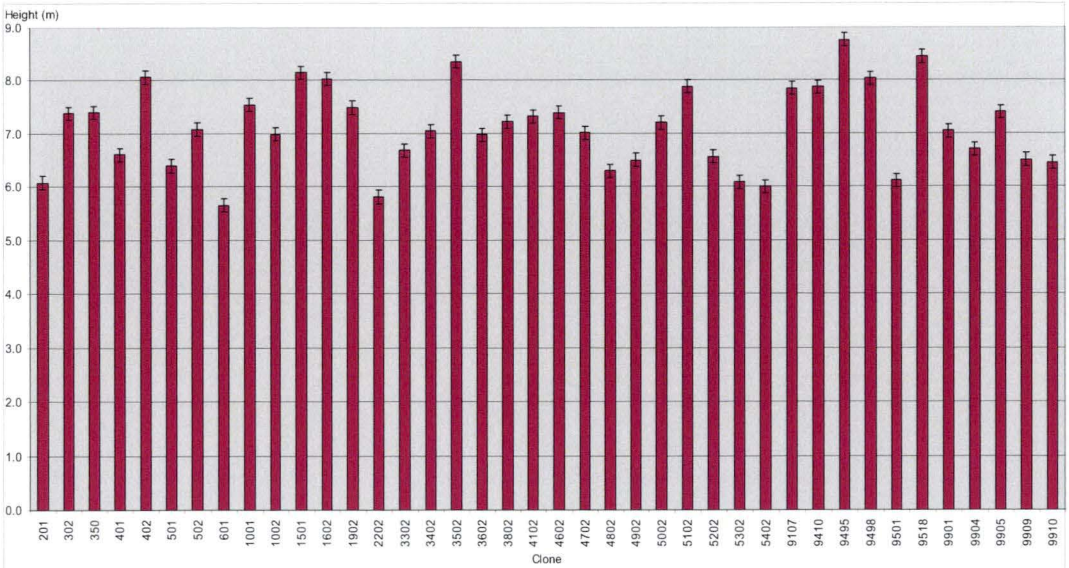


Figure 3.2.1: The average heights of clones and landrace (9909) at Song May trial. The bar denotes standard error.

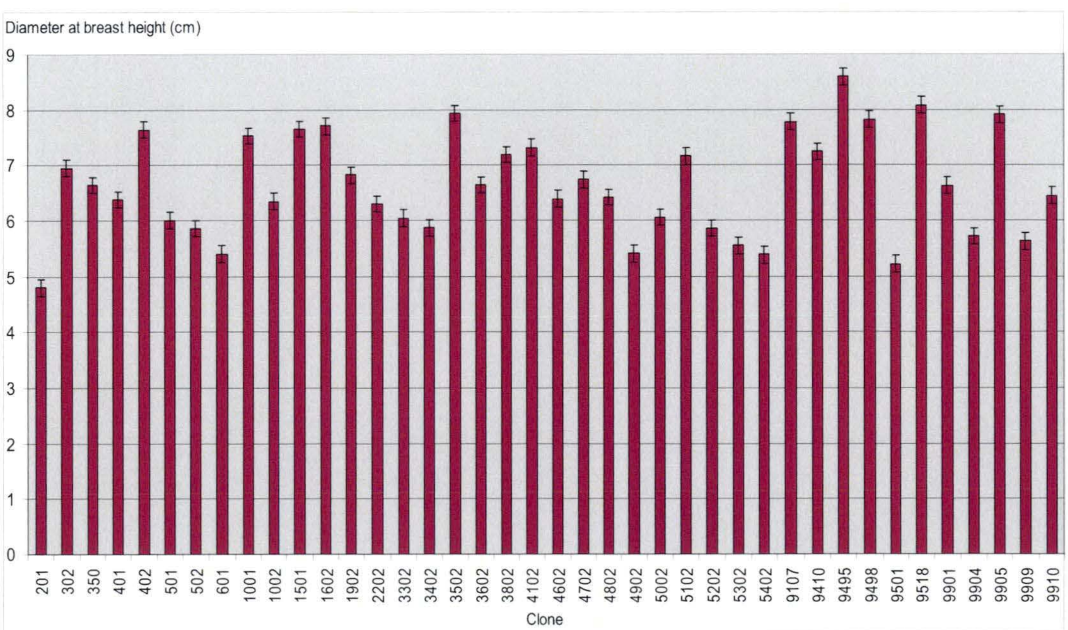


Figure 3.2.2: The average diameters at breast height of clones and landrace (9909) at Song May trial. The bar denotes standard error.

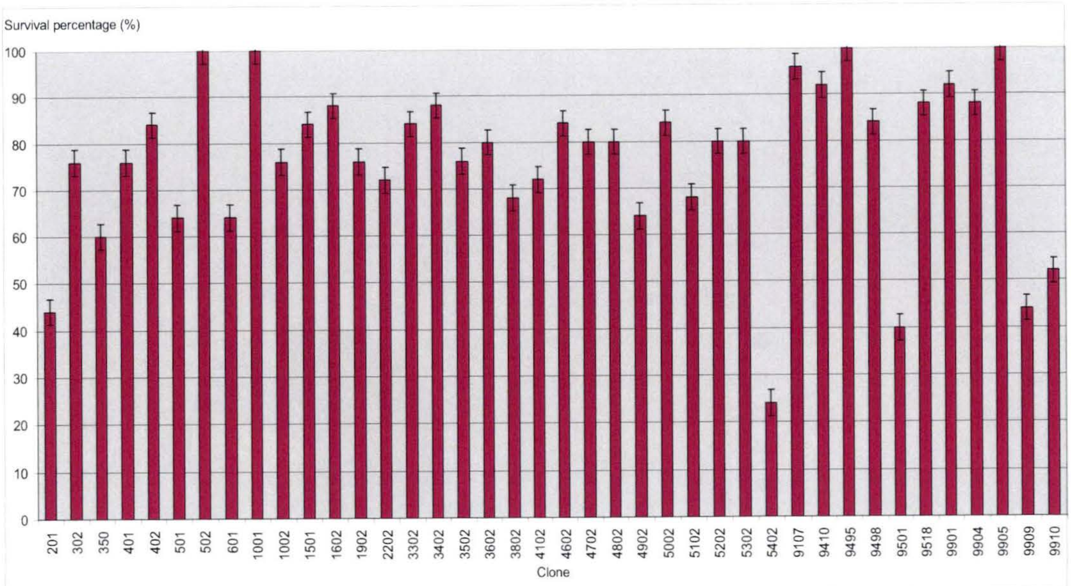


Figure 3.2.3: The average survival rates of clones and landrace (9909) at Song May trial. The bar denotes standard error.

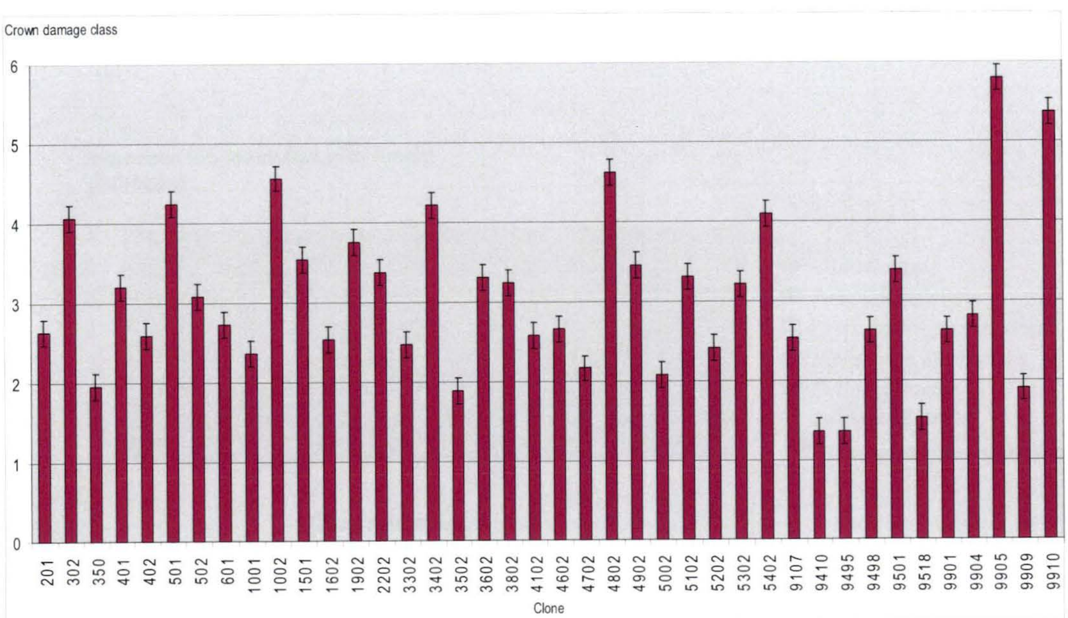


Figure 3.2.4: The average crown damage indices of clones and landrace (9909) at the Song May trial. The bar denotes standard error.

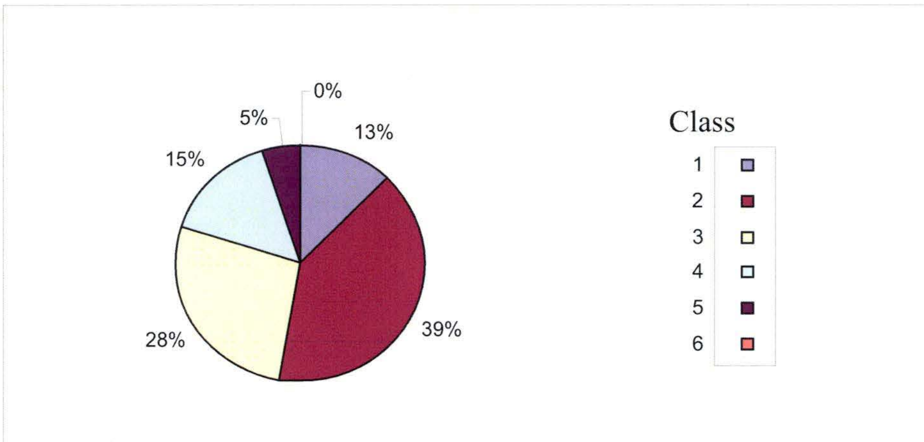


Figure 3.2.5: Crown damage index at Song May site; class 1 is a healthy crown, class 6 is a severely damaged crown, classes 2-5 increase stepwise in the severity of crown damage.

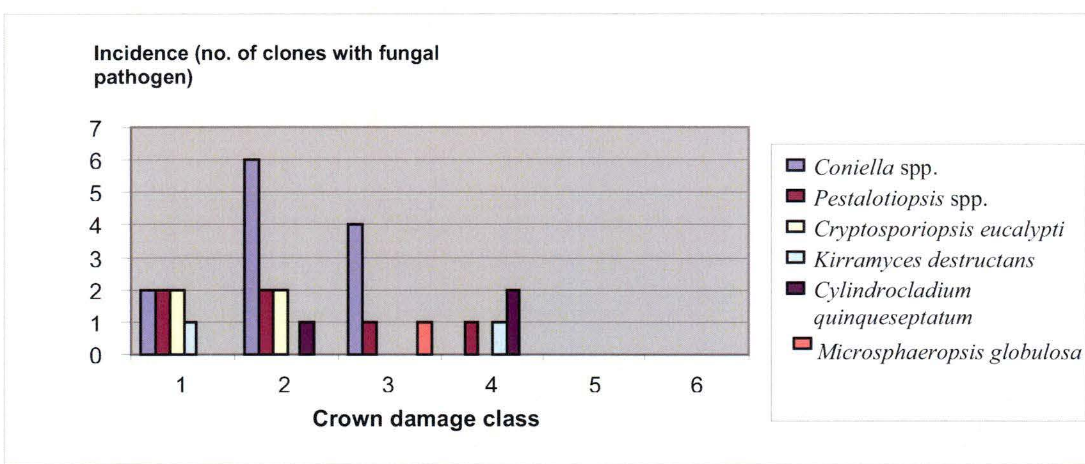


Figure 3.2.6: Incidence of fungal pathogens in each of 6 severity classes at Song May.

3.3. Bau Bang site (10 clones)

Height: Clone mean heights were ranged from 6.3 to 10.1 m (Fig.3.3.1). On average, clone mean height was 8.2 m. There were six clones, including the landrace control with mean heights below the mean point (i.e. 60% of the clones planted on site). Out of these six clones three (9501, 9905 and 9911) had heights significantly lower (p=0.001) than the mean point and were therefore the poorest performers in respect to height.

There were four clones with mean heights above mean point representing 40% of the total number of clones on site. Clones 9495 and 9518 had significantly greater (p=0.001) mean heights than other clones on site (Appendix 1.3.1) and were the best performing clones in respect to height. Average mean height of these best clones was 10 m with a growth rate of 30.4 cm per month.

Diameter at breast height: Clone mean diameters ranged from 4.5 to 8.1 cm (Fig.3.3.2). Average mean clone diameter was 6.7 cm. The mean diameters of five clones fell below the average mean clone diameter i.e. 50% of all clones tested at this site. Three of these 5 clones (9498, 9501 and 9911) were significantly smaller (p=0.001) than those clones with mean diameters above the mean point.

There were five clones (50% of the total number of clones on site) with diameters above the mean point. Out of these 5 clones, clones 9906 and 9495 were significantly different (p=0.001) and markedly larger in diameter than clones falling below the mean point (Appendix 1.3.1). The average mean diameter of these two clones was 7.8 cm and they were clearly the best performing clones on site in terms of diameter.

Survival: Survival rates for clones were between 62.5 and 98.8% (Fig.3.3.3). The average mean survival rate for the clones was 89.1%. There were three clones with survival rates below the mean point for survival rate i.e. 30% of the total number of clones on site. Clone 9501 and landrace control (9909) had significantly lower rates (p=0.001) of survival compared to clones with survival rates above the mean point.

There were seven clones with survival rates higher than clone average mean point for survival rate. Three clones 9495, 9905 and 9498 (ranked from 1 to 3) had significantly higher rates ($p=0.001$) of survival than the other six clones of trial and had survival rates of 98.8%.

Crown damage index: Scores for crown damage at this site ranged between classes 1 and 6 but there were no clones scored in class 5 (mean clone crown damage indices ranged from 1.2 to 6.0 (Fig.3.3.4)). Average mean clone crown damage index was 3.3.

Five out of ten clones were scored as or above class 3 (one as class 6, one as class 4 and two as class 3). Four clones fell above the mean point for crown damage indices. Only three of these 4 clones (9498, 9905, 9906) had significantly higher levels of crown damage ($p=0.001$) compared to the other seven clones.

Five clones (half of those on site), including the control landrace, had crown damage scores lower than the mean point of 3.3 (two clones were class 2 and 3 clones were class 1 (Fig 3.3.5)). Only two of these 5 clones (9495, 9518,) and the control landrace (9909) had significantly lower ($p=0.001$) crown damage indices than the other seven clones on site with very low crown damage scores of 1 (Appendix 1.3.1).

Fungal pathogens (Appendix 1.4)*:* Clone 9495 (scored as class 1) was infected by *M. globulosa* and *Coniella* species. *C. fragariae* and *K. destructans* were both present in the landrace control but the damage cause by these fungi was low (class 1). *C. fragariae* was present at a low level in clone 9518 (class 1). *C. australiensis* was associated with class 2 damage in clone 9911.

Clone 9107 (class 3) was infected by both *Coniella* sp. and *M. globulosa*, clone 9501 (class 3) was damaged by *C. fragariae*. Damage to clone 9498 in class 4 was associated with both *C. australiensis* and *M. globulosa*. The most severe damage in clones 9905 and 9906 (class 6) was associated with *K. destructans*. Clone (9912) at this site was diseased by fungal pathogens, but these pathogens could not be definitively identified.

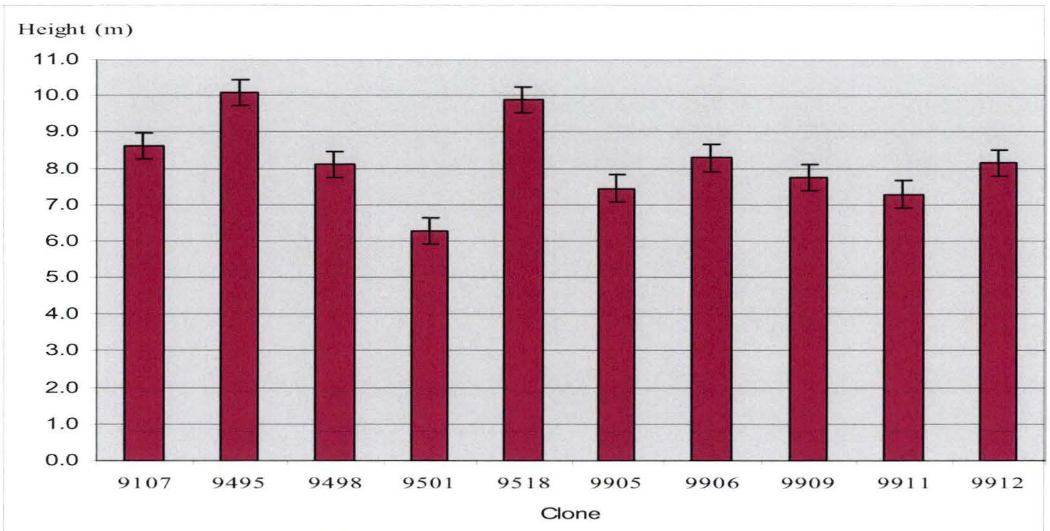


Figure 3.3.1: The average heights of clones and landrace (9909) at Bau Bang trial. The bar denotes standard error.

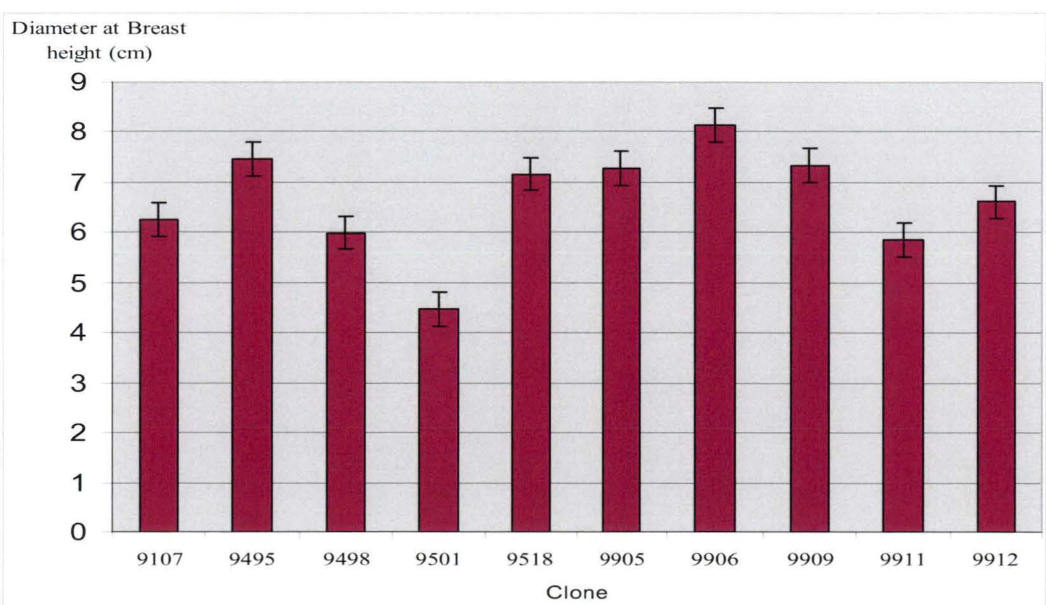


Figure 3.3.2: The average diameters at breast height of clones and landrace (9909) at Bau Bang trial. The bar denotes standard error.

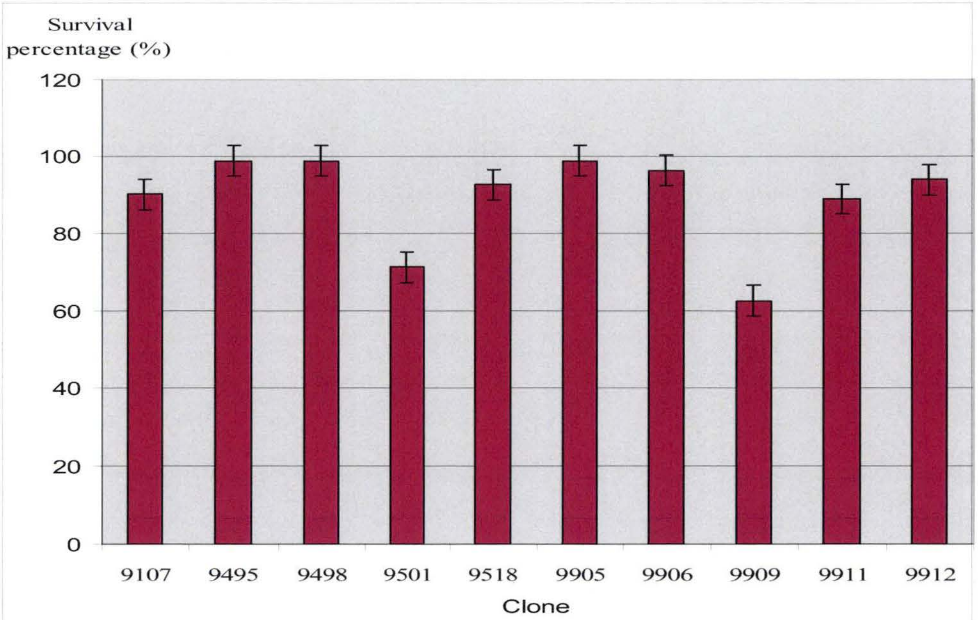


Figure 3.3.3: The average survival rates of clones and landrace (9909) at Bau Bang trial. The bar denotes standard error.

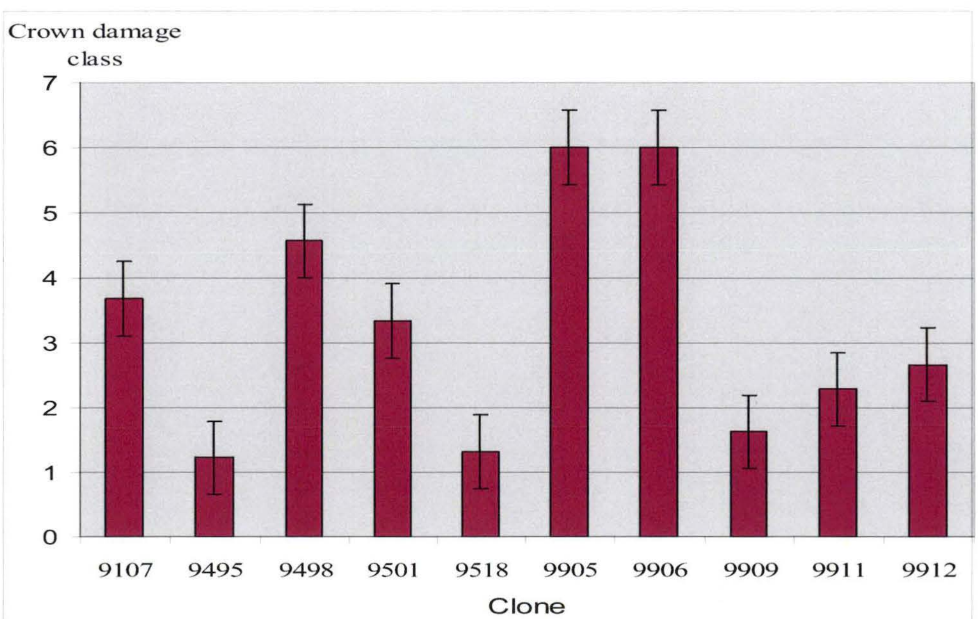


Figure 3.3.4: The average crown damage indices of clones and landrace (9909) at Bau Bang trial. The bar denotes standard error.

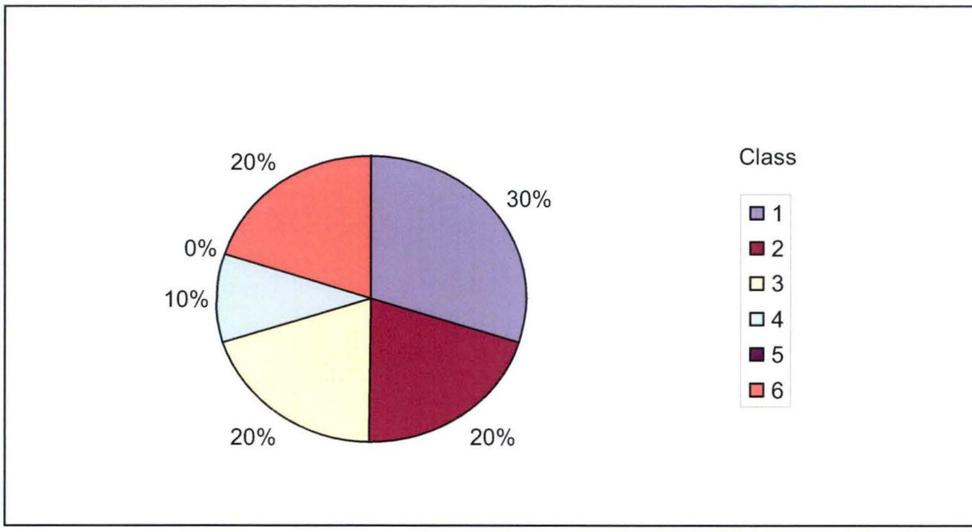


Figure 3.3.5: Crown damage index at Bau Bang site; class 1 is a healthy crown, class 6 is a severely damaged crown, classes 2-5 increase stepwise in the severity of crown damage.

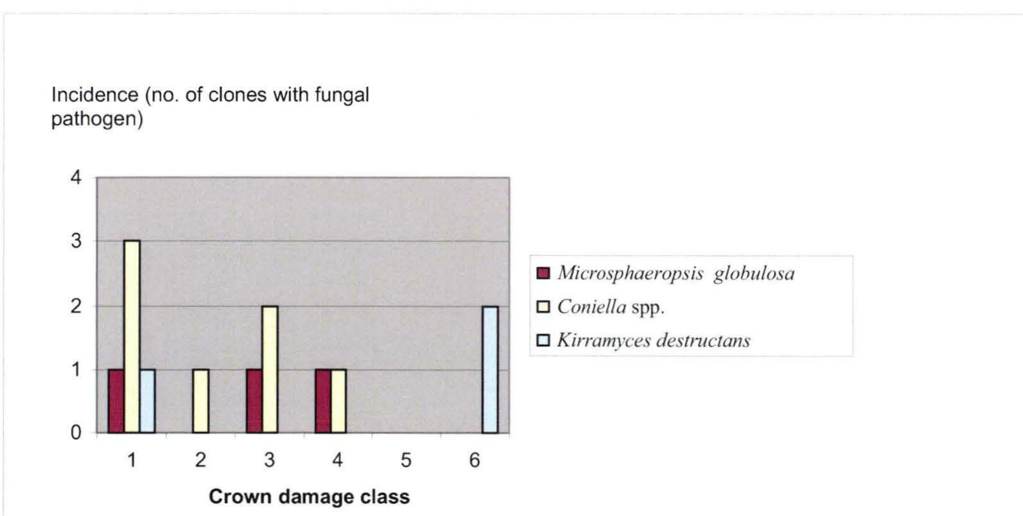


Figure 3.3.6: Incidence of fungal pathogens in each of 6 severity classes at Bau Bang.

Summary of results at Minh Duc:

Based on the four criteria of height, diameter, survival ability, and crown damage index:

- The growth and survival of the landrace control was poor at this site although its crown damage index was better than average.
- Clones that can be considered as less vigorous and/or susceptible to disease are 201, 401, 402, 601, 701, 902, 2002, 4602, 4802, 5202, 5402, 6402, 9908 and 9907 (14 clones, accounting for 31.8% of the total number of clones).
- Among those clones for which pathogens were identified, *Coniella* spp. were the most common agent causing crown damage, observed on twenty-three clones or 52.3% of the total number of clones at the site. The incidence of *C. eucalypti* was very low but severity in this case was high (one clone was scored at class 4). Overall disease incidence at Minh Duc site was low.
- The best performing clones (in terms of disease resistance and growth) are 9903, 9518 and 9902. Clone 9495 is also considered a good performer at this site. Its height was not significantly different to that of other clones but it satisfied the other two selection criteria, including resistance to disease.

Summary of results at Song May:

- The growth and survival rate of the landrace control was relatively poor at this site but was highly resistant to disease.
- Based on height, diameter, survival ability, and crown damage index, the following clones, grew poorly with low survival rates and/or high susceptibility to disease: 201, 302, 501, 601, 1002, 2202, 3402, 4802, 4902, 5302, 5402, 9501, 9904, 9905 and 9910 (i.e. 16 clones including control landrace or 40% of the total number of clones at the Song May site).
- At this site there were four types of fungal agents associated with the highest levels of crown damage and disease; species of *Pestalotiopsis*, *Coniella*, *C. quinqueseptatum* and *K. destructans*. The incidence of species of *Coniella* at this site was relatively high (it was identified from 12 clones) but the severity of

disease caused by *Coniella* was low and it was mainly associated with crown damage scores of 1 and 2

- Clones 9495 and 9518 were the best performing clones at the Song May site in terms of growth and disease resistance.

Summary of results at Bau Bang:

- The survival and growth of the landrace control was relatively poorly at this site but it appeared very resistant to disease.
- Based on height, diameter, rate of survival, and crown damage index, the following clones appeared to have the lowest vigour and survival rates and/or greatest susceptibility to disease: 9501, 9498, 9911, 9905, 9906 and the landrace control (six clones i.e. 60% of the total number of clones on site).
- The incidence of *Coniella* spp. at this site was high as it was found in seven clones but the severity of associated damage was variable (four clones in classes 1-2, three clones in classes 3-4). *M. globulosa* was found on three clones; the highest level of damage with which it was associated was class 4. *K. destructans* was observed on three clones and was associated with severe damage (class 6) to two of these clones.
- Clone 9495 was the best performing clone on site in terms of growth and disease resistance. Clone 9518 was not a top performer in terms of diameter but performed very well in respect to disease resistance, survival rate and height.

3.4. Combined site analysis of clonal performance

In the combined site analysis, the main effects of site and clone were significant for height and crown damage index ($p<0.05$) when tested against the site.clone mean square. For survival, the site effect was significant ($p=0.018$) but not the clone effect ($p=0.819$) (Appendix 1.3.3). This means that sites differed significantly in terms of height, survival rate and crown damage index; clones differed in terms of height and crown damage index but not survival. However, the site-by-clone interaction was significant when tested against the pooled residual mean square for height ($p<0.001$), survival ($p<0.001$) and crown damage index ($p<0.001$) (Appendix 1.3.2 and

Appendix 1.3.3) indicating that there were significant changes in the ranking of clones across the different sites for all three traits.

Poor clonal performance in the combined site analysis

The combined analysis showed that nine of the 60 clones and control landrace were significantly ranked as poor performers in terms of height ($p<0.001$) (Fig.3.4.1, Table 3.1.4). The analysis also showed that there were nine clones which had significantly lower survival rates ($p<0.001$) than the other 60 clones and control landrace (Fig.3.4.2, Table 3.4.1). In respect to crown damage index, ten clones scored significantly higher or were more diseased ($p<0.001$) than other clones (Fig.3.4.3 and Table 3.1.4).

Overall, in the combined site analysis 18 out of 60 clones (29.5%) and the control landrace performed poorly in terms of height, survival rate and crown damage index; they were included among 29 clones assessed in individual site analyses as poor performers (Table 3.1.4).

Of these 18 poorly performing clones, eleven clones were tested at a single site, six clones were tested at two sites and only one clone (9905) was tested at all 3 sites (Table 3.4.1). Of the 11 clones tested at a single site, all of them were ranked as poor performers in the individual site analysis. Of the six clones tested at two sites, five clones (201, 601, 4802, 5402 and 9501) were ranked as poor performers in terms of height and/or survival and/or crown damage index. Clone 9905 (a hybrid between *E. urophylla* and *E. camaldulensis*) tested at all 3 sites was susceptible to disease as indicated by high crown damage indices at two sites (Song May and Bau Bang) and moderate disease resistance at Minh Duc.

Table 3.4.1: Details of poorly performing clones at each of the three trial sites and combined site analysis; x denotes a trait which significantly indicates poor performance (p=<0.001). Five clones (in bold) were poor performers. 9909 is landrace control.

Site	**Minh Duc**				**Song May**				**Bau Bang**				**Combined site analysis**		
Clone	H (m)	Dgl (cm)	Sur[1] (%)	CDI[2]	H (m)	Dbh (cm)	Sur[1] (%)	CDI[2]	H (m)	Dbh (cm)	Sur[1] (%)	CDI[2]	H (m)	Sur[1] (%)	CDI[2]
201	x	x			x	x	x						x	x	
302								x							
401	x	x		x									x		
402			x												
501								x					x		x
601	x	x	x		x	x							x	x	
701	x	x	x	x									x	x	x
902				x											x
1002								x							
2002			x											x	
2202					x								x		
3402								x							
4602			x												
4802		x		x	x			x							x
4902						x									
5202				x											
5302					x	x							x		
5402	x	x	x		x	x	x	x					x	x	
6402				x										x	x
9498										x		x			
9501					x	x	x		x	x	x		x	x	
9904						x									
9905								x	x			x			x
9906												x			x
9907	x		x	x										x	x
9908				x											x
9909						x	x				x				
9910							x	x						x	x
9911									x	x					

[1]: Sur = survival ; [2]: CDI = crown damage index

Superior clonal performance in the combined site analysis

The combined site analysis showed that nine clones (ranked from 1 to 9 out of 60 clones) and the control landrace were significantly different ($p<0.001$) in terms of height (Fig.3.4.1). Six had significantly higher survival rates ($p<0.001$) and were ranked 1 to 6 out of 60 clones and control landrace (Fig.3.4.2). In terms of crown damage index eleven had significantly lower scores or less disease ($p<0.001$) than the other 60 clones and control landrace (Fig.3.4.3) with crown damage indices ranging from 1.5 to 2.0 (they were ranked lowest i.e. 50 to 61).

All together, the combined site analysis showed that four clones or 6.6% of the 60 clones and control landrace (9495, 9903, 9518 and 9902) were the best performing clones in terms of height, survival rate and crown damage index (Table 3.4.2). They were also the best performing clones in the single site analyses.

Of these 4 superior performing clones, clones 9495 and 9518 were tested at all three sites and were superior to all other clones (Table 3.4.2). The two other clones, 9902 and 9903, tested at a single site (Minh Duc) also exhibited significantly superior performance (Table 3.4.2).

Table 3.4.2: Details of superior clones at each of the three trial sites and combined site analysis; x denotes a trait which significantly indicates superior performance (p=<0.001), y denotes a crown damage index in class 1. Four clones (in bold) were good performers. 9909 is landrace control.

Site	Minh Duc				Song May				Bau Bang				Across site		
Clone	H (m)	Dgl (cm)	Sur[1] (%)	CDI[2]	H (m)	Dbh (cm)	Sur[1] (%)	CDI[2]	H (m)	Dbh (cm)	Sur[1] (%)	CDI[2]	H (m)	Sur[1] (%)	CDI[2]
305								x							x
402					x	x									
502							x							x	
1001		x		x		x	x								
1302													x		
1501	x	x		x	x	x							x		
1602					x	x							x		
2702			x	y											
3402	x			x											
3502					x	x		x					x		
3802			x												
4402		x	x	y											
4702															x
5002															x
5402				y											
7301	x			y									x		
9107						x	x								
9410				y				x							x
9495		x		y	x	x	x	x	x	x	x	x	x	x	x
9498	x				x	x					x				
9518	x	x	x	x	x	x		x	x			x	x	x	x
9901														x	
9902	x	x		y									x	x	x
9903	x	x	x	x									x	x	x
9904				x											
9905	x	x				x	x				x				
9906										x					
9909								x				x			x
9911															x
9912															x

[1]: Sur = survival; [2]: CDI = crown damage index

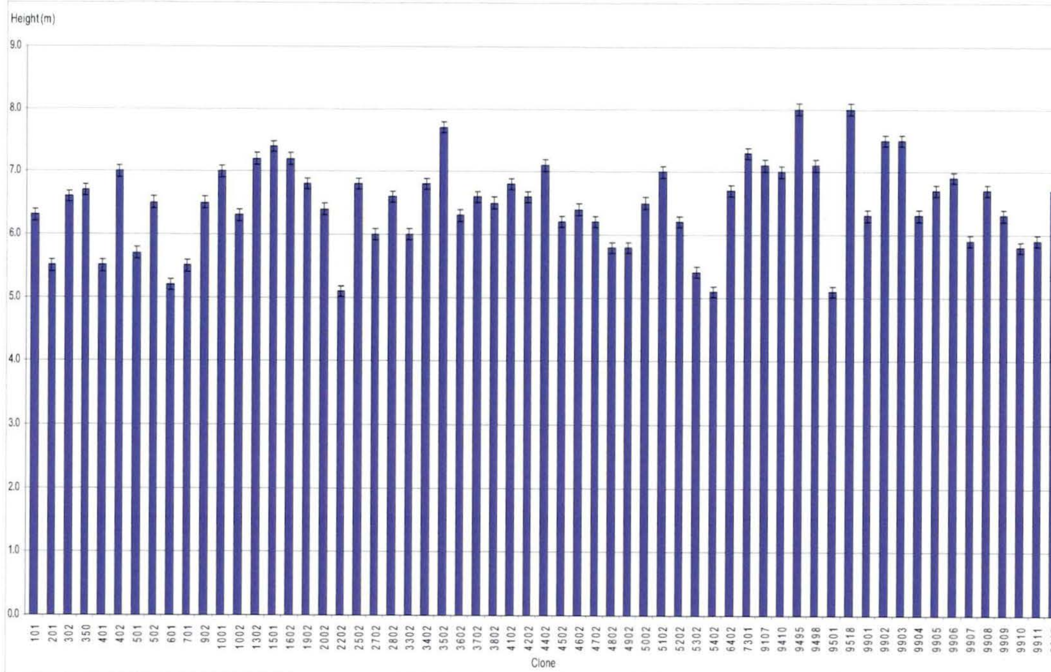


Figure 3.4.1: Predicted means for height (m) of clones and landrace (9909) in the combined site analysis. The bar denotes standard error.

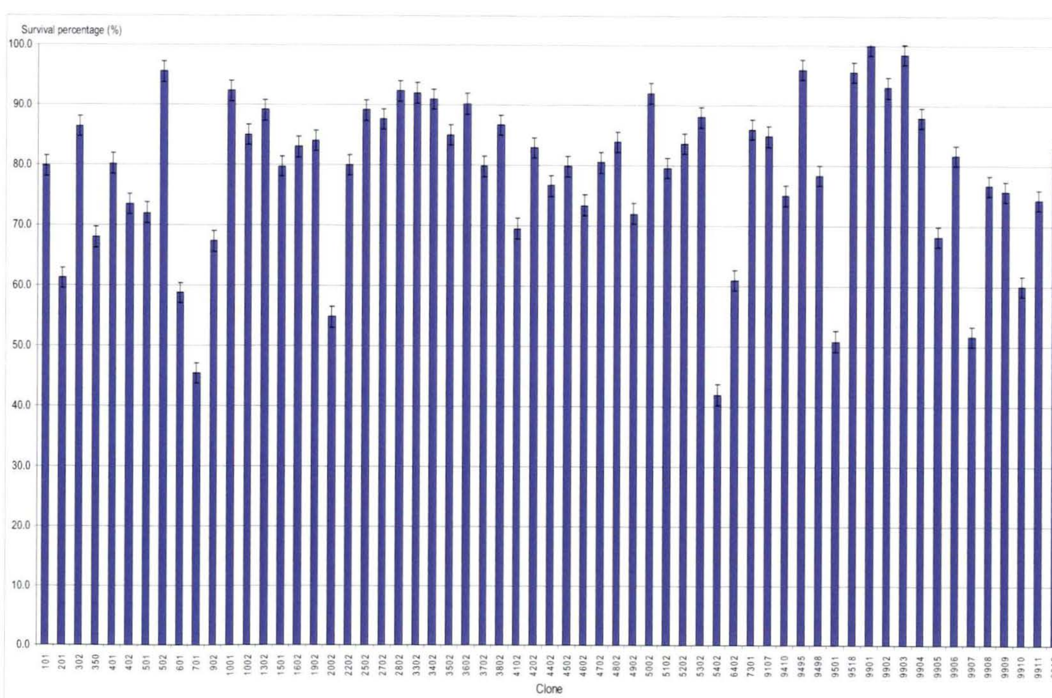


Figure 3.4.2: Predicted means for survival rate (%) of clones and landrace (9909) in the combined site analysis. The bar denotes standard error

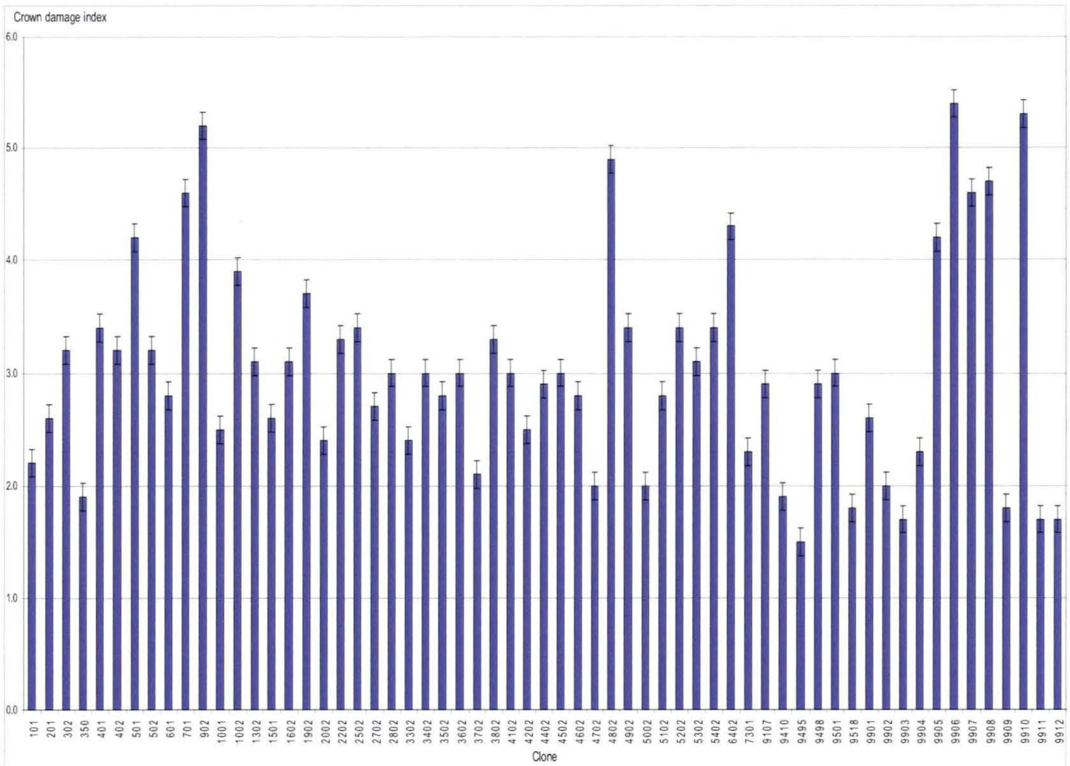


Figure 3.4.3: Predicted means for crown damage index of clones and landrace (9909) in the combined site analysis. The bar denotes standard error

Inconsistencies in the ranking of clones in the combined site analysis

Some clones planted at more than one site were not consistent across sites in their performance or in terms of the particular fungi associated with damage. In addition, the combined site analysis did not always adequately reflect the results of individual site analyses.

✓ Two clones and the control landrace, each tested at three sites, behaved differently at these sites.

⇨ **Clone 9498** (*E. camaldulensis*) performed very well in terms of growth at Minh Duc and Song May but poorly at Bau Bang (Table 3.4.1 and 3.4.2). This clone was ranked among those with moderate resistance to *C. fragariae* (crown damage index was 2.2) at Minh Duc and an unidentified fungal species (crown damage index was 2.6) at Song May. However, at Bau Bang, it proved susceptible to *C. australiensis* and *M. globulosa* (crown damage index was 4.6). In the combined site analysis, this clone was ranked with others as not being susceptible to disease.

⇨ **Clone 9905** (*E. urophylla* x *camaldulensis*) grew very well at Minh Duc and Song May and also had a high survival rate at Song May and Bau Bang but it performed poorly in terms of height at Bau Bang. It was among clones with moderate resistance to *C. fragariae* (crown damage index was 2.6) at Minh Duc but it was very susceptible to an unidentified fungal disease (crown damage index was 5.8) at Song May and susceptible to *K. destructans* (crown damage index was 6.0) at Bau Bang. The combined site analysis indicated that this clone was significantly susceptible to disease compared to all other clones (Table 3.4.2).

⇨ **The control landrace** (*E. camaldulensis*) had comparatively low survival rates at Song May and Bau Bang and performed poorly in terms of growth at Song May. This landrace showed moderate disease resistance to an unidentified fungal species (crown damage index of 2.1) at Minh Duc but was significantly resistant to *K. destructans* and *C. fragariae* at Song May and Bau Bang. The combined analysis also showed that the control landrace was significantly resistant to disease.

✓ Five clones, each tested at two sites, behaved differently at these sites.

⇨ **Clone 3402** (*E. camaldulensis*) grew well at Minh Duc and exhibited resistance to *C. eucalypti* and *Coniella* spp. (crown damage index was 1.3). In contrast, at Song May, its growth was not as good as at Minh Duc and it proved susceptible to an unidentified fungal disease (crown damage index was 4.2). In the combined site analysis, it ranked with clones not susceptible to disease.

⇨ **Clone 6402** (*E. camaldulensis*) was ranked among clones with a moderate survival at Song May and among clones with poor survival at Minh Duc. The combined analysis classed clone 6402 among clones with moderate survival rate.

- ⇨ **Clone 4702** (*E. camaldulensis*) ranked among clones with moderate resistance to diseases at Minh Duc and Song May but the combined site analysis showed that it was significantly resistant to disease.
- ⇨ **Clone 5402** (*E. camaldulensis*) performed poorly in terms of growth and survival rate at both Minh Duc and Song May and the combined site analysis reflected showed those results. It was resistant to disease (*Coniella* leaf spot with crown damage index of 1.7) at Minh Duc whereas very susceptible to *C. quinqueseptatum* (crown damage index of 4.1) at Song May. However, this clone was ranked as not susceptible to disease in the combined site analysis.
- ⇨ **Clone 9904** (*E. tereticornis*) was among clones with moderate resistance to an unidentified fungal species at Song May (crown damage index was 2.8) but significantly resistant to an unidentified fungal species at Minh Duc (crown damage index was 1.4) (Table 3.4.1 and 3.4.2). In the combined site analysis, it was ranked among clones with moderate resistance to disease.

✓ The ranking in the combined site analysis of five other clones tested each at a single site did not reflect the results of the individual site analysis for these clones.

- ⇨ **Clone 501** (*E. tereticornis*) was among clones with moderate height performance at Song May but it was classed as a significantly poor performer in terms of height by the combined site analysis.
- ⇨ **Clone 1302** (*E. camaldulensis*) was among clones with moderate height performance at Minh Duc but the combined analysis ranked it as a significantly superior performing clone in terms of height.
- ⇨ **Two clones (5002, and 9912)** were among clones with moderate resistance to diseases at Song May (5002) and Bau Bang (9912), but the combined site analysis showed that they were significantly resistant to disease.
- ⇨ **Clone 9911** (*E. tereticornis*) showed poor performance in terms of growth at Bau Bang and it was among clones with moderate disease resistance (crown damage index was 2.3) at this site. In the combined site analysis, this clone was shown to be significantly resistant to disease (Table 3.4.2).

Incidence of fungal pathogens across the three sites

Coniella leaf spot was the fungal pathogen with the highest incidence for all three sites (observed on 42 clones) but its severity was generally low (classes of 1 and 2, Table 3.4.3) Only four clones assessed as class 4 had damage associated with species of *Coniella*. In general, as crown damage indices increased the damage was less likely to be associated with *Coniella* spp. *Cryptosporiopsis eucalypti*, *Pestalotiopsis* or *Cylindrocladium quinqueseptatum* caused the greater severity of damage. Species of *K. destructans* and *M. globulosa* were the most damaging species recorded. The clones infected by these species were scored in classes 6 and 5 respectively (Table 3.4.3).

Table 3.4.3: Incidence of fungal pathogens at all 3 sites.

		No. of clones in each crown damage class						
Fungal pathogen	Site	1	2	3	4	5	6	*Total*
Coniella spp.	Minh Duc	10	8	2	3	-	-	*23*
	Song May	2	6	4	-	-	-	*12*
	Bau Bang	3	1	2	1	-	-	*7*
	Total	*15*	*15*	*8*	*4*	-	-	***42***
Cryptosporiopsis eucalypti	Minh Duc	1	1	-	1	-	-	*3*
	Song May	2	2	-	-	-	-	*4*
	Total	*3*	*3*	-	*1*	-	-	*7*
Kirramyces destructans	Song May	1	-	-	1	-	-	*2*
	Bau Bang	1	-	-	-	-	2	*3*
	Total	*2*			*1*		*2*	***5***
Pestalotiopsis spp.	Song May	2	2	1	1	-	-	***6***
Cylindrocladium quinqueseptatum	Song May	-	1	-	2	-	-	***3***
Microsphaeropsis globulosa	Song May	-	-	-	1	-	-	*1*
	Bau Bang	1	-	-	1	1	-	*3*
	Total	*1*	-	-	*2*	*1*	-	***4***
Unidentified fungal pathogen	Minh Duc	7	8	2	3	1	-	*21*
	Song May	1	5	6	2	2	-	*16*
	Bau Bang	-	1	-	-	-	-	*1*
	Total	*8*	*14*	*8*	*5*	*3*	-	***38***

4. Discussion

The study has assessed and described three clonal eucalypt trials in southern Vietnam with four criteria that are height, diameter, survival rate and crown damage index. It is recognised that this study only presents the results from a single observation of young trees and that further assessments must be carried out. Among the 60 clones and the control landrace tested, nearly half of them were associated with measurements of one or more factors indicating poor growth (including height and diameter), low survival rate or disease susceptibility. There were only a limited number of clones that performed well for all criteria – growth, survival and apparent disease resistance. The surveys in thesis also identified prevalent and significant fungal pathogens. These pathogens though were present as a result of the vagaries of natural infection and its assessment which makes it difficult to unequivocally attribute the absence of disease with host resistance e.g there was very little disease at the Minh Duc site which could have been due to lower rainfall during the wet season and/or assessment before maximum expression of disease. However early good growth is a significant component of final productivity and when combined with absence of disease these early results do strongly indicate the potential of various clones for futher testing. Future testing of promising clones could be carried out by artificial inoculation with serious pathogens to back up the results of the field testing of resistance.

a) Disease susceptibility associated with poor growth or vigour

Among 29 clones which were considered as poor performers in terms of growth, there were up to sixteen clones which appeared more susceptible to disease (e.g. two poorly growing clones (701, 9907) at Minh Duc were very susceptible to disease with crown indices of over 4). Two other clones (401, 4802) also exhibited poor growth and proved susceptible to several fungal diseases including *Cylindrocladium quinqueseptatum* (crown damage indices over 4.5). Although disease susceptibility appears linked with poor growth criteria, it is difficult to clarify the nature of the relationship between tree vigour and susceptibility. Indeed clones 9905 and 9906 were superior in terms of growth in this study but appeared very susceptible to fungal pathogens. Disease resistance per se may not relate directly to vigour but the latter is still a useful secondary character when selecting superior clones for regions subject to high infection.

b) Comparison of performance when clones grown at more than one site

There was a very limited number of clones (5) grown at all three sites and only a third of the total number of clones was planted at two sites. Because of the large number of missing values, the estimated values for the combined site analysis (Results 3.4, Appendix 1.3.2) were inconsistent with the individual site analyses in the ranking of clones according to the different criteria. There was however, some real variation in the performance of clones across sites that were highlighted by the combined site analysis.

Variation in performance of a clone in our study is illustrated by clone 9498 (*Eucalyptus camaldulensis*). It had, prior to this study, already undergone fairly intense selection in trials at Hue and Dai Lai, central and north Vietnam respectively (Nghia 2003, 2006). It was earmarked as a clone with good growth and disease resistance. In our study, this clone – tested at all 3 sites - showed a marked variation in its performance. It was relatively disease resistant at both sites; its growth was very good at Song May and moderate at Minh Duc. It grew poorly however at Bau Bang and proved very susceptible to *Coniella australiensis* and *Microsphaeropsis globulosa*. Other clones such as 3402 showed a similar variation in behaviour, growing well and resistant to disease at Minh Duc but more susceptible to disease at Song May.

It is important to assess the early potential shown by clone(s) in terms of how they performed both when grown at the different sites in this study and when previously grown at other locations in Vietnam.

Hybrid clone 9907 (*E. urophylla* x *camaldulensis*) was originally selected by the Phu Linh Paper research Centre in Phu Tho province as having high vigour and disease resistance to fungi when screened in northern Vietnam (Phu Tho, Hoa Binh, and Bac Giang provinces; Fig.2.1.1, Nghia 2004, 2006). It was promoted as an advanced clone by the Ministry of Forestry of Vietnam (currently known as the Ministry of Agricultural and Rural Development). This hybrid clone was then shown to be highly susceptible to the wilt bacterium, *Ralstonia solanacearum* (Fig.A1.4.8) in trials in northern Vietnam (Nghia 2003, 2006). In this study's trials in southern Vietnam, it also proved susceptible to fungal disease.

Clones 9905 and 9906 (hybrid clones of *E. camaldulensis* x *urophylla* imported from China) had been carefully selected from previous trials in the central and northern regions of Vietnam, Hue and Dai Lai respectively (Fig.2.1.1) and considered as advanced varieties in terms of growth and disease resistance by the Ministry of Agriculture and Rural development of Vietnam (Nghia 2003, 2006). Clones 9905 and 9906 were also superior in terms of growth in this study in southern Vietnam but appeared very susceptible to fungal pathogens. Trees of these clones were severely infected by *Kirramyces destructans* with crown damage indices of 6.0.

The results of this study indicate that clones do not always perform in the different regions of Vietnam - or even in the same region when planted at different sites. This will be due to a complex of interacting factors such as site productivity, climate and the presence of suitable conditions for disease epidemics (i.e. sufficient pathogen inoculum, a susceptible host and favourable conditions for natural infection). This variation in performance indicates a requirement to carry out a large number of spatially and temporally separate trials before a clone can be considered as one of superior performance. Potential clones from this trial that have not already been tested e.g. clones 9905 and 9906 must be screened at sites in northern, central and southern Vietnam. The screening should be for all insect and disease pests of significance, e.g. bacterial wilt that caused 30% mortality in young eucalypt plantations of clone 9907 in Phu Tho and Bac Giang and even up 50% in similar eucalypt plantings in Vinh Phuc (Thu *et al.* 2000; Nghia 2003, 2006). *R. solanacearum* attacks many kinds of eucalypt hosts, such as *E. amaldulensis*, *E. grandis*, *E. pellia*, *E. urophylla* and (Ciesla *et al.* 1996) and it is widespread throughout tropical, subtropical and warm temperate regions (Smith *et al.* 1992) such as in Indonesia (Machmud 1985), Thailand (Pongpanich 2000), South Africa

(Coutinho *et al.* 2000), Australia (Akiew and Trevorro 1994) and Brazil (Dianese *et al.* 1990).

c) Good performance at more than one site

Clones 9495 and 9518 grew better than all other clones at all three sites. They were highly resistant to *Coniella* leaf spot, *Pestalotiopsis* sp., *Cryptosporiopsis eucalypti*, and even *M. globulosa* (the latter two were potentially the most damaging fungal pathogens in this study). Clones 9495 and 9518 are considered as advanced varieties by the Ministry of Agriculture and Rural development of Vietnam (Nghia 2006). In Nghia's experiment with clonal eucalypt trials in Dai Lai, northern Vietnam, planted in 2003 and in Binh Dien (Hue province), planted in 2002, clones 9495 and 9518 also exhibited superior growth and disease resistance (Nghia 2004, 2006). At a eucalypt trial planted in 1998 at Song May, these clones also performed well and were highly resistant to disease (Nghia 2006). As a result of this study and other corroborating evidence from previous Vietnamese trials, clones 9495 and 9518 can be fairly confidently planted in Vietnam at any environment as they show superior performance. A caveat exists in that this situation could change due to a new disease incursion or changes in existing pathogen populations.

d) Resistance to foliar fungal pathogens

Eucalypts have many foliar diseases caused by a wide range of fungi. In Australia, foliar diseases are common in native eucalypt forest but only sporadically destructive. Fungal diseases have proved a problem in eucalypt plantations both in Australia and countries where eucalypts are exotic such as Vietnam. There are many examples of destructive epidemics in eucalypt plantations in Australia, New Zealand, South Africa, Brazil and India (Purnell and Lundquist 1986; Crous *et al.* 1989; Sankaran *et al.* 1995; Pegg *et al.* 2003; Dick *et al.* 2006). Outside Australia e.g. Vietnam, destructive epidemics of diseases maybe caused by local fungi (new encounter pathogens), successful new encounter pathogens in one region that travel to other geographical localities and coevolved pathogens introduced from Australia.

Eucalypt species were first planted in the 1930's in Vietnam (Thiep 1996). The major foliar fungal pathogens known in tropical eucalypts have been identified and reported from Vietnam, apart from *Puccinia psidii* (Old *et al.* 2003; Glen *et al.* 2007a). However there are probably new encounter pathogens present in the plantations that have not been identified or their pathogenicity ascertained (especially as eucalypt diseases in the tropics are often caused by non specialist fungal diseases with a wide host range such as *Cylindrocladium* spp. and *Erythricium salmonicolor* (pink disease)).

Coniella spp. have been reported in Australia, South Africa, India, Israel, Russia, Papua New Guinea, France, Netherlands, Norway, Hawaii, Vietnam, Thailand and Indonesia (Sharma 1994; Yuan 1996; Pegg *et al.* 2003; Andrianova and Minter 2005). These species have been reported attacking the hosts of eucalypts such as *E. grandis*, *E. pellita*, *E. tereticornis*, *E. urophylla* (Pegg *et al.* 2003).

In Vietnam, *Coniella* species normally infect the lower part of a eucalypt crown, the older leaves (Nghia 2006) and are rarely associated with damage to the entire crown. In our study, *Coniella* leaf spot (caused by two species – *C. australiensis* and *C. fragariae* (Old *et al.* 1999)) were the most prevalent fungi causing disease symptoms at all three sites. Although in the majority of cases *Coniella* spp. were associated with the senescent part of the crown, the level of damage attributed to this fungal pathogen could be high and over the entire crown. Clones were also infected by *Coniella* spp. in combination with other fungal pathogens such as *C. eucalypti*, *M.globulosa* and *K. destructans*. Multiple infections of the same tree by different pathogens have been frequently observed in Vietnam (Nghia 2000; Old *et al.* 1999). The level of damage observed in our study where *Coniella* spp. appeared to act as a primary pathogen or were present in multiple infections demonstrates the potential of this pathogen to cause crown damage especially to young eucalypts in Vietnam.

Leaf spots caused by *Cryptosporiopsis eucalypti* can occur on both juvenile and adult leaves, and cause severe defoliation and dieback of young shoots (Ciesla *et al.* 1996; Old *et al.* 2002). *C. eucalypti* has been reported from Australia, New Zealand, Sri Lanka, Hawaii, India, Indonesia, Japan, Laos, Thailand, as well as Vietnam (Old and

Yuan 1994; Sankaran *et al.* 1995; Gadgil and Dick 1999; Old *et al.* 2000b). In Vietnam, species of *C. eucalypti* (and *Cylindrocladium quinqueseptatum*) have been described as the most damaging fungi to eucalypts causing defoliation, shoot death or crown deformation or dieback or even stem cankers (Sharma 1994; Booth *et al.* 1999; Old *et al.* 2000b; Old 2001; Nghia 2003, 2006).

In this study, there were 7 clones which were diseased by *Cryptosporiopsis eucalypti*, but only one clone (9908 – eucalypt species not known) at Minh Duc was severely diseased by this fungus. Even though *E. camaldulensis* has been reported as relatively susceptible to *C. eucalypti* (Old *et al.* 2003b; Thu 2005a) clones of this species, in this study, were not severely diseased by this fungus. No clones of *E. brassiana* or its hybrids were visibly infected by this species. *E. tereticornis* has been observed in previous studies to be moderately resistant to *C. eucalypti* (Old *et al.* 2002, 2003b). Our results reflect those of Old as *C. eucalypti* infected only one clone (201) of *E. tereticornis*. *E. brassiana* and *E. tereticornis* should be used in planting when it is suspected that site conditions favour the development of *C. eucalypti*.

At the end of 1980s, epidemics of *Cylindrocladium quinqueseptatum* leaf blight in the southern and central provinces of Vietnam caused severe defoliation and volume loss in *E. camaldulensis*, *E. urophylla*, and *E. deglupta* (Booth *et al.* 2000; Thu *et al.* 2000). Lower levels of damage to eucalypts were observed in the northern provinces of Vietnam (Thu *et al.* 2000). Booth *et al.* (2000) used climate mapping to identify high-risk areas for *C. quinqueseptatum* leaf blight on eucalypts in Vietnam. Booth *et al.* showed that high-risk areas in Vietnam are locations with a minimum mean annual rainfall of 1400 mm and a mean minimum temperature for the coldest month of 16 °C. Certain regions of Vietnam were thus determined as being at high risk to *C. quinqueseptatum* - such as the north-east and north of Ho Chi Minh City, parts of the Hue region (Fig.2.1.1). Regions indicated as low disease risk were in Vinh region (about 200 km far to the north of Hue) and other parts of northern Vietnam.

All of the three sites described in this thesis fell in high-risk areas according to Booth *et al.* (2000) but *C. quinqueseptatum* was recorded at only one site, Song May, on 3 clones. Two of these clones infected by *C. quinqueseptatum*, had relatively high scores. *C. quinqueseptatum* was therefore present at the Song May site and capable of causing crown damage to *E. camaldulensis* clones but it appears that many of the clones were resistant to this pathogen. At the other two sites either the pathogen was not present and/or conditions were unsuitable for disease development and/or the clones were relatively resistant and inoculum load as kept at a low level.

Other authors have also found inconsistencies in the level of attack by this pathogen. Dudzinski *et al.* (1999) when assessing a eucalypt trial in Chon Thanh (Fig.2.1.1) showed a low incidence and severity of *C. quinqueseptatum* leaf blight. Old visited Minh Duc and Bau Bang trials in 2004 and did not detect Cylindrocladium leaf blight (pers. comm.). It is likely that over time we are now observing a general shift towards resistance to this disease in both the routine plantings of eucalypts and, as would be expected, in clones in genetic trials e.g. Minh Duc and Bau Bang in this study. Another example of this shift towards resistance is the landrace control in our study (used for routine planting of *E. camaldulensis* in Vietnam). It showed poor growth and survival at both Song May and Bau Bang but the crowns were healthy and apparently highly resistant to disease at all three trial sites. This landrace has been widely planted in Vietnam and although it has not been specifically selected for high vigour it may have undergone a degree of natural selection in terms of resistance to *C. quinqueseptatum* (and other major fungal eucalypt pathogens) in Vietnam.

Fungal pathogens will also adapt to less favourable conditions presented by their environment and/or host resistance especially if there is the opportunity for sexual recombination and variation. Booth *et al.* (2000) showed that eucalypts growing at higher cooler elevations in Vietnam are less vulnerable to *C. quinqueseptatum* (telemorph: *Calonectria quinqueseptata*). For example, *C. quinqueseptatum* has been found at high cool altitude (at Dalat, Fig.2.1.1) in a nursery situation (*E. saligna* seedlings) but not in the surrounding eucalypt plantations (Thu *et al.* 2000). However, in 2002, *C. quinqueseptatum* was recorded in eucalypts in northern Vietnam in Tan Lac, Hoa Binh province although with a relatively low level of damage (Nghia 2006). Since this region is outside of the disease risk area as mapped by Booth (1996) (using the VIET climatic mapping program) this observation may indicate that there has been selection pressure on the pathogen and it has adapted to a previously marginal environment.

Microsphaeropsis globulosa was recorded in Vietnam for the first time in eucalypts in this study. Incidence of this fungal pathogen was low but when it did occur it caused serious damage to the eucalypt host. Microsphaeropsis leaf spot has been recorded in Tasmania on *E. obliqua* as *M. callista* (Yuan 1999). This fungus *M. globulosa* has also been reported from Argentina, South Africa and Sri Lanka (Old *et al.* 2003b). *M globulosa* should be given more attention in Vietnam as it clearly has potential to cause damage on eucalypts.

Another very significant fungal pathogen, *K. destructans,* was found at Song May and Bau Bang. *K. destructans* is an aggressive and often devastating pathogen that causes distortion of infected leaves and blight of young leaves, buds and shoots (Wingfield *et al.* 1996). This pathogen was first discovered in Indonesia in 1996 and has subsequently spread to Thailand, China, and East Timor (Old *et al.* 2003a; Old *et al.* 2003b; Burgess *et al.* 2006). It has been recently found in Northern Territory, Queensland and Western Australia (Mohammed pers. comm.). In 2002, *K. destructans* was first recorded as being present in several locations in northern, central and southern Vietnam (Old *et al.* 2003a; Nghia 2003); it was recorded as causing moderate damage to *E. camaldulensis*, *E. urophylla* in eucalypt plantations and nursery seedlings in southern provinces (Old *et al.* 2003b; Nghia 2006). In this study, two clones at Bau Bang (9905 and 9906) were severely damaged by this fungus and were the most damaged clones at the trial. Nghia (2006) also reported clones 9905 and 9906 as infected with *K. destructans* in northern Vietnam. Given its history of severe damage in other countries this pathogen should be given high priority in resistance screening and risk prediction.

Although the above-mentioned fungal leaf and shoot blight pathogens have caused significant damage in Vietnam, resistance screening must also consider other types of potentially damaging fungal pathogens in Vietnam. For example, *K. epicocoides*, *Pseudocercospora eucalyptorum* and *Mycosphaerella marksii* that cause leaf diseases; *Cryphonectria cubensis*, *C. gyrosa*, *Lasiodiplodia theobromae*, *Coniothyrium zuluense* and pink disease which cause stem cankers. Although, in Vietnam, these fungal pathogens cause lower levels of damage to eucalypts and impact less on the growth they are often observed in eucalypt plantations and nurseries (Thu 2002, 2005b; Nghia 2006). There are also insect pests of concern e.g. *Leptocybe invasa* Fisher & La Salle. This insect pest is believed native to Australia (Mendel *et al.* 2004; FAO 2007). It is an example of a pest that has moved out from Australia, originally to Israel and then to many other countries Uganda, Israel, Italy, Jordan, Morocco, Spain, Kenya, New Zealand, Turkey including Vietnam (Mutitu 2003, Mendel *et al.* 2004; Nyeko 2004; Thu 2004; Nyeko *et al.* 2007). This pest species has been recorded on more than 10 *Eucalyptus* species (e.g. *E. camaldulensis*, *E. globulus*, *E. gunii*, *E. grandis*...) and is a high priority pest for most countries growing eucalypts as an exotic species. A biological control has been released in Israel and is being investigated in other countries.

As the eucalypt plantation estate expands in Vietnam there will be new opportunities for the adaptation, spread and introduction of pathogens and pests especially if restrictions on the movement of germplasm are not enforced within Vietnam and for entry into Vietnam.

e) Application of the lessons learnt in the molecular study of wood decay fungi (see Section 2 of this thesis) to the detection and identification of fungi from eucalypts in Vietnam

A list of the major fungal pathogens that could be expected on eucalypts grown in Vietnam can be found in Old *et al.* 2003b.

Known leaf and shoot blight pathogens include species of *Meliola, Cryptosporiopsis, Cylindrocladium, Mycosphaerella, Kirramyces, Coniella, Microsphaeropsis, Aulographina* and *Pseudocercospora.* All these fungi vary greatly in their taxonomy, morphology and physiology and in the degree of information publicly available especially in respect to molecular profiles e.g.

- ✓ *Aulographina eucalypti* - No sequences available
- ✓ *Coniella australiensis* - One ITS and one LSU (large sub-unit of the ribosomal RNA) sequence available (Van Niekerk *et al.* 2004)
- ✓ *Coniella fragariae* LSU only, no ITS sequence
- ✓ *Cryptosporiopsis eucalypti* - No sequences available but ITS sequences available for 6 other *Cryptosporiopsis* species.
- ✓ *Cylindrocladium* - ITS sequences for 19 species are available in GenBank (Crous 2002)
- ✓ Canberra cultures of *Cylindrocladium* sp. include VN76-82
- ✓ *Meliola* - No ITS sequences available
- ✓ *Microsphaeropsis* - No ITS sequences available
- ✓ *Mycosphaerella* – over 50 species are known from eucalypts – most have sequences from multiple gene regions available, but new species are still being discovered (Crous *et al.* 2006) and not all species are clearly distinguished by ITS sequences, e.g. *M. vespa, M. ambiphylla* and *M. molleriana* (Glen *et al.* 2007b). Therefore, sequencing of additional gene regions may be necessary to discriminate to species level.
- ✓ *Kirramyces destructans, epicoccoides, eucalypti* ITS sequences available for all 3 species.
- ✓ *Pseudocercospora* - ITS sequence from three isolates of *P. eucalyptorum* are available; as are ITS sequences from many other *Pseudocercospora* spp.

A similar strategy as adopted for the wood decay fungi in this study would be highly relevant to developing tools for the molecular detection and identification of major pathogens direct from eucalypt leaf tissue in Vietnam. However if only the major fungal pathogens causing damage are targeted then the procedure might be less complicated with fewer limitations as encountered when trying to identify an extremely wide suite of fungi from rotting wood. The steps involved are as follows:

- ✓ Intensive surveys of fungi associated with eucalypt leaves and shoots in Vietnam. Taxonomic descriptions of any un- or poorly described fungi that appear to cause serious damage (NB morphological expertise in fungal identification must be maintained at a high level and molecular methods used as complementary tools to assist in identification procedures).

- ✓ Isolations from reproductive structures to obtain fungal cultures of the major fungal agents that appear to cause visible damage.
- ✓ DNA sequencing of cultures and phylogenetic analyses.
- ✓ Development of specific primers for major pathogens and testing of their specificity against a wide suite of fungal cultures such as might represent endophytic fungi in a leaf or saprophytic fungi.
- ✓ Development of multiplex nested PCR (e.g. Glen *et al.* 2007b) for the major eucalypt foliar and shoot fungal pathogens present in Vietnam.

e) Management of fungal diseases

The management of large scale plantations will continue to intensify in Vietnam involving the deployment of clonally propagated highly productive genotypes, environmentally sustainable land preparation, use of fertilisers, the application of disease control strategies, particularly the selection of disease resistant genotypes. The selection of disease resistant clones is considered the best tactic for the management of disease in eucalypt plantations where clonal reproduction is possible (Gadgil *et al.* 2000). Several authors (Blum *et al.* 1992; Nghia and Old 1997; Sharma *et al.* 1999; Old *et al.* 2003b) have shown it is possible to select for resistance in eucalypts especially against certain pathogens such as *C. eucalypti* and *C. quinqueseptatum* leaf blight - and that this is the best and most successful route in Vietnam. Our study has also shown that there are some potentially resistant and vigorous clones (e.g. 9495, 9518). A caveat to the development of clonal resistance is that a wide selection of clones are deployed in such a way to avoid selection pressure on pathogens and pests. Matching specific species, hybrids and their clones to a particular site risk for a disease or pest may be one strategy to adopt.

5. Conclusions

This study described growth and crown damage assessments for three clonal eucalypt trials planted in southern Vietnam. Some clones exhibited both poor growth and disease susceptibility e.g. 401, 701. Other clones were assessed as fast growing and highly resistant to disease e.g. 9495, 9518. Some significant fungal pathogens such as *Kirramyces destructans*, *Cylindrocladium quinqueseptatum,* species of *Coniella,* and *Microsphaeropsis globulosa* were present at the trials and clearly capable of causing damage; there were however excellent examples of clonal resistance to these diseases at the various sites. This experiment did not assess growth and resistance criteria in clonal eucalypt trials that were planted in very diverse environments. However by reference to previous results obtained by other authors, certain clones showed a site /environmental related susceptibility to disease, e.g. clone 9498 was susceptible to disease when it was tested in the south of Vietnam but resistant to disease when it was planted in the north.

The selection of species with high yield and resistance to disease is a long and complicated process requiring research in several different areas;

- ✓ There were many fungi present in the trials that could not be identified. A baseline of fungi present in eucalypt plantations should be established in Vietnam and an assessment made of their pathogenicity. More investment is required in building a capacity in classical fungal taxonomy.
- ✓ It is very important to study the influence of several generations of genetic selection on eucalypt - pathogen interactions; fungi have a capacity to adapt to changes in host and may be undergoing selection for pathogenicity.
- ✓ It is necessary to investigate the mechanisms of tree resistance to diseases as an aid to programmes for resistance breeding/selecting.
- ✓ The field screening trials are however the most important component to these programs and should be set up to evaluate the complete range of environmental factors that might influence the progress of a disease epidemic. It is possible that a particular combination of environmental conditions will shift one of these

relatively minor (and most often unidentified) fungi to cause significant levels of damage.

Molecular tools (as described in the following chapter) can be used to assist in all the above studies e.g. identifying fungal pathogens and/or studying their population genetics. The use of molecular detection and identification of fungal pathogens direct from tissue and at asymptomatic stages of development is particularly applicable to investigating the ecology and epidemiology of fungal pathogens on different hosts at different sites.

Section 2: The development of molecular tools to detect and identify fungal pathogens

1. Introduction

1.1. Rationale for developing detection tools for fungi in rotting wood material

Wet eucalypt forest is a widespread forest type in Tasmania, comprised of approximately 883000 ha of the 3.17 million ha of native forest in the state (Bureau of Rural Science 2005). Eucalypts in this system include *E. obliqua, E. regnans* and *E. delegatensis* (Kirkpatrick 1988).

Wood decay fungi are commonly associated with woody rot. In the forest ecosystem, wood decay fungi play an important role in the cycling of carbon and nitrogen (Grove *et al.* 2002; Heilmann-Clausen and Christensen 2003), while helping to convert organic debris into the humus layer of the soil (Jasalavich *et al.* 2000; Hoff *et al.* 2004; Allmér *et al.* 2006). Some fungi damage living trees, others occupy dead or dropped logs and slash on the forest floor (Hoff *et al.* 2004). Research has been carried out over the past decade in these forests in southern Tasmania to investigate the fungi and insects present in living trees and logs on the forest floor in order to inform forest management in respect to biodiversity and conservation values (Yee 2005; Hopkins 2006). These authors showed that beetles were linked to specific rot types either in the veteran trees or large diameter logs and specific fungi were also associated with certain types of rotting wood. There were no evident three-way associations among beetles, fungi and rot types.

For this type of research to be continued successfully and due to the considerable site to site variation there is a requirement for the large scale sampling of the fungi associated with rot types. A DNA-based method to detect and identify the occurrence of wood decay fungi would potentially use only small amounts of wood, thus allowing for non-destructive sampling with wood corers of logs compared to laborious chain saw dissections (Jasalavich *et al.* 2000). Accurate, rapid, and cost-effective molecular tools provide a valuable method to differentiate fungal associations and have the potential for detecting multiple fungal species simultaneously occupying the same niche (Glen *et al.* 2007a). Many fungal species that grow in wood are not culturable under laboratory conditions (Johannesson and Stenlid 1999). Therefore, isolation results may not give a true representation of the fungi present in tissue. Of those that are culturable, most basidiomycetes can not be artificially induced to yield fruiting bodies which are critical characters for morphological identification of genera, families, and species (Nobles 1948).

However the taxonomy of wood decay fungi in Australia is poorly developed (May 2001; Hood 2003; Simpson 1996) and therefore we do not have reliable information to develop molecular detection tools for wood decay fungi. Only 5% of the probable number of species of fungi in Australia are classified and described in detail (Hawksworth 1991; May and Pascoe 1996). This is mainly due to the shortage of professional mycologists and the short history of mycological research in Australia (Hopkins 2006). Therefore the taxonomy of wood decay fungi is based mainly on outdated literature of Australian fungi (e.g. Cunningham 1965).

To assist fungal identification and study the relationships among fungal groups, a molecular tool called the Polymerase Chain Reaction (PCR) can be used to amplify specific fragments of DNA (Hoff *et al.* 2004). The ribosomal DNA internal transcribed spacer (rDNA ITS) is widely considered to be an appropriate region for fungal species discrimination as inter-specific species variation in the ITS of many fungal species has been observed (Mitchell *et al.* 1995; Kårén *et al.* 1997; Farmer and Sylvia 1998; Glen *et al.* 2001; Horton and Bruns 2001; Hopkins 2006). The level of intra-specific heterogeneity does vary between species, though, and some intra-generic homogeneity has also been observed (Glen *et al.* 2001). The conserved nature of the subunits surrounding the ITS (18S, 5.8S and 28S) has also made the development of universal fungal primers possible for phylogenetically diverse group of fungi (White *et al.* 1990; Hoff *et al.* 2004). As the ITS region is widely used for fungal studies, it is well suited for identification of unknown fungal isolates as it is possible to compare their ITS sequences with those currently deposited in public electronic databases (e.g. Genbank, EMBL, and DDBJ). Public sequence databases (GenBank, EMBL, DDBJ) have several shortcomings for fungal identification.

Australian fungi, for example, are under represented and there is rarely any ability to verify the species identification given as the source of the sequence.

Molecular identification will never replace the requirement for morphological expertise in funal identifications. However, robust molecular tools for the detection and identification of fungi direct from plant tissue are highly desirable (whether these are wood decay fungi or foliar pathogens of eucalypts) but certain problems have to be overcome before this tool can be developed and it is necessary to follow a certain strategy to ensure success.

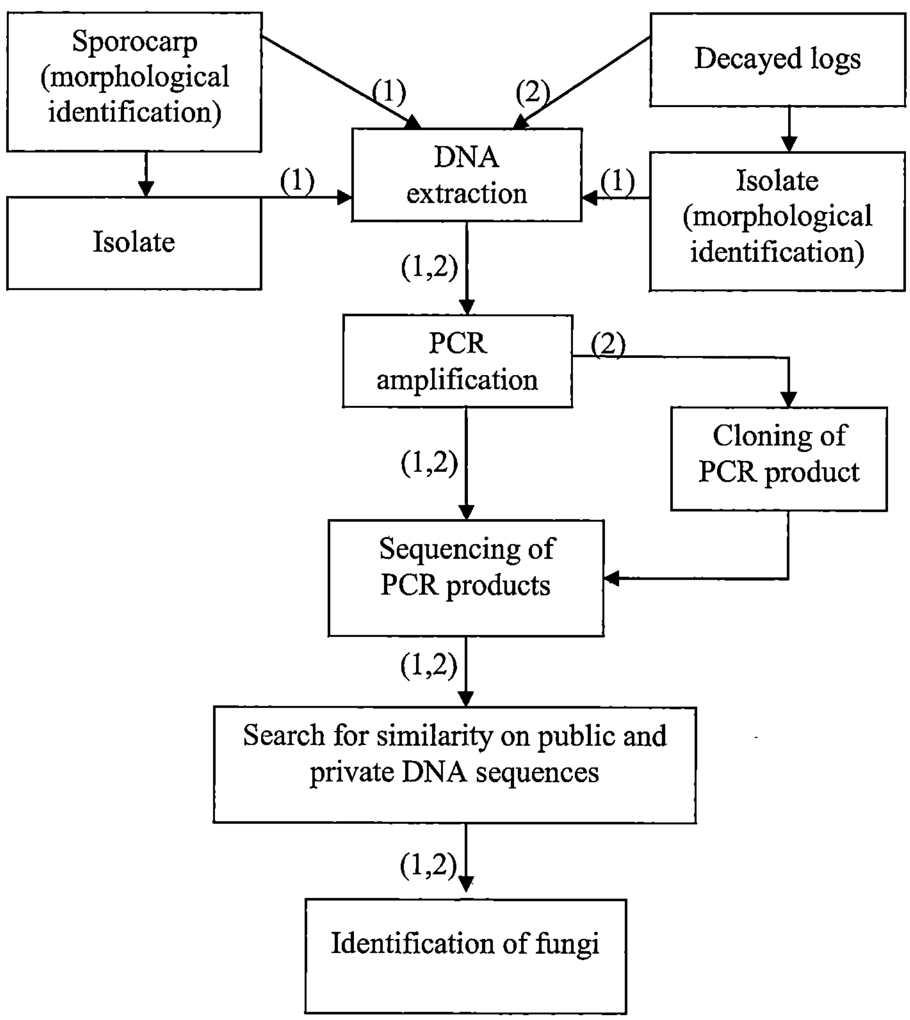


Figure 1.2.1: Diagram for establishing reference sequences and identifying fungi from decayed log material.

This chapter therefore aims to follow this strategy (Fig.1.2.1) towards developing a molecular tool for the identification and detection direct from wood. The steps involved are: (i) identification of fungi directly from sporocarps (ii) identification and matching of fungi from reference isolates and isolates from sporocarps (iii) detection and identification of fungi directly from decayed material taken from logs.

2. Materials and methods

2.1. Sample collection, morphological identification of sporocarps and isolations obtained from sporocarps

2.1.1. Collection and processing of sporocarps

To form a reference collection of identified sporocarps, with cultures and ITS sequences linked to these sporocarps, ninety-six sporocarps were collected from a number of different locations in Tasmania on an opportunistic basis (Appendix A2.1 and A2.2.1) These sporocarps were dried and stored at the CSIRO/Ensis herbarium in Hobart, Tasmania. In addition, fifteen sporocarps (*Ganoderma* spp.) were collected in the Northern Territory on various hosts (Appendix 2.1) and were preserved in a 70% ethanol solution.

A clean, 0.125 cm^3 piece of each sporocarp was removed and stored in an eppendorf tube at -20 ^{o}C to be used for DNA extraction. All sporocarps (except for the *Ganoderma* isolates preserved in alcohol) were then described and air dried according to the instructions in Brundrett *et al.* (1996).

2.1.2. Morphological identification of sporocarps

Eighty-four out of a hundred and eleven sporocarps were morphologically identified to species or genus levels with the assistance of a mycologist (Genevieve Gates). Twenty-seven sporocarps were not identified to either genus or species level (Table 3.1.1.1 to Table 3.1.4).

2.1.3. Fungal cultures obtained from sporocarp

Isolations to obtain fungal cultures were attempted from the ninety-six sporocarps that had not been preserved in alcohol. No isolations were obtained with twenty-six of the sporocarps. Successful isolations were obtained from seventy sporocarps. The method followed Hopkins (2006). Briefly: 10-15 small pieces of sporocarp from within the cap or stipe were excised. These pieces were then sterilised for 1 minute in 95% ethanol, then for 1.5 minutes in 5% domestic White King bleach (approximately 2.5% available chlorine) and incubated for four to six weeks on MAT (1% malt, 20 g agar, 950 ml water, 50 ppm penicillin, 50 ppm streptomycin, 25 ppm polymyxin and 230 ppm thiabendazole) at 20 °C. An isolation procedure was considered successful when the majority of sporocarp pieces produced morphologically similar cultures. This mycelium was then subcultured and maintained on MA (1% malt, 20 g agar, 1000 ml water) at 20 °C for the duration of the study. Copies of each isolate have been stored in the Ensis/CSIRO Forest Health culture collection in Hobart.

The mycelium was harvested from 14-21 day old cultures and stored in an eppendorf tube at -20 °C to be used for DNA extraction. To easily remove mycelium from media, cultures were grown on MA medium covered with sterilized cellophane.

2.2. Wood decay isolates and samples of rotted wood

2.2.1. Rotted wood sample collection

Samples of rotted wood were collected during a previous study. This study was based on decayed woods and fungal isolates collected by Yee and Yuan (Yee 2005) from *E. obliqua* logs in the wet scelrophyll forest of southern Tasmania. Yee and Yuan collected the decayed wood samples from logs at seven sites in two different forest types; three sites in mature, unlogged forest and four sites in logging regenerated forest (Table 2.2.1). For more detailed site descriptions, see Yee (2005), Sections 2.2 and 2.3. At each site, three small diameter logs (30-60 cm) and three large diameter logs (>100 cm diameter) of *E. obliqua* at an intermediate decomposition stage (defined by Yee (2005), Section 2.5) were sampled. Details of the study logs were listed in Yee (2005), Table 2.2. Samples of the different types of rotten wood were removed, placed in an airtight bag, and taken for more comprehensive laboratory descriptions. These samples of rotten wood were labelled corresponding to the map drawn of each disc (Yee 2005, chapter 4). Representative samples of rotten wood types were kept for reference in a cool room after the study had been completed.

Table 2.2.1: Site location, recent disturbance history and forest coupe code (reproduced from Yee (2005), only the sites relating to this study are shown in this table).

Forest type	Site code and access Road	Latitude	Longitude	Year of clearfelling	Year of last wildfire
Logging regenerated forest	E Edwards Rd	43.0918 S	146.7473 E	1969	-
	S South weld Spur 1	43.0826S	146.7223E	1975	-
	W Warra Rd	43.0872S	146.7186E	1975	-
	H Hartz Rd	43.1601S	143.8021E	1966	-
Mature unlogged forest	M Manuka Rd	43.0933S	146.6442E		1906
	WR Manuka Rd	43.0935S	146.713E	1983	1914

2.2.2. Isolates obtained from decayed wood

Immediately after collection small pieces of rotten wood samples representative of different rot types were incubated by Yuan (Z.Q. Yuan unpublished data) on MA or MAT to isolate any associated fungi. The resulting 762 isolates of wood decay fungi obtained were sorted into 63 major morphospecies and 77 ungrouped isolates, based on their macro-and microscopic characteristics (Mohammed and Yuan 2002).

For this study 114 isolates were selected to represent the range of morphotypes distinguished by Yuan. These were subcultured onto MA medium for DNA work but 21 of them did not grow or were contaminated. Ninety-three isolates were successfully subcultured. Of those 93 isolates, twenty-seven isolates had been identified to species or genus levels, based on their cultural morphology, thirty-two isolates were grouped into twenty-two groups based on a comparison of cultural morphology and thirty-four isolates were ungrouped. Mycelium was harvested from 14-21 day old cultures of each of subculture and stored in an eppendorf tube at -20 °C to be used for DNA extraction.

2.3. DNA extraction, PCR amplification and sequencing of i) sporocarps, ii) fungal cultures obtained from sporocarp and iii) isolates obtained from decayed wood

DNA extraction of sporocarp tissues and mycelia was carried out using the glassmilk method described by Glen *et al.* (2002). Briefly, sporocarp tissue, approximately 100 mg, was ground under liquid nitrogen by using a plastic pestle; fresh mycelium, approximately 100 mg, was ground with a motorised pestle in a 1.5 ml microcentrifuge tube. 250 µl extraction buffer (Raeder & Broda 1985) was added to the material and mixed with the ground material before incubation at 65 °C for 60 minutes. The tubes were centrifuged at 14,000 rpm for 15 minutes. DNA was purified by binding to glassmilk. The supernatant (200 µl) was removed and added to a fresh tube containing 800 µl of cold 100% (w:v) NaI and 7 µl glassmilk. The mixture was vortexed briefly then incubated on ice for 15 minutes with occasional shaking, to allow the DNA to bind to the glassmilk. Tubes were centrifuged at 14,000 rpm for 10 seconds, the supernatant discarded, and the pellet was resuspended in 800 µl of wash buffer (10 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl in 50% ethanol). Following centrifugation at 14,000 rpm for 10 seconds, the supernatant was removed and the pellet resuspended in 800 µl 100% ethanol. After a final centrifugation, the supernatant was discarded and the pellet was completely air dried in a laminar flow cabinet. DNA was eluted by adding 25 µl TE buffer (10 mM Tris HCl pH8, 1 mM EDTA), vortexing briefly and incubating at 45 °C for 5 minutes. Supernatant containing DNA was removed to a new 1.5 ml centrifuge tube following centrifugation at 14,000 rpm for 2 minutes, and stored at -20 °C.

A total of 274 DNA samples were extracted, in which DNA was extracted from each of 111 sporocarp samples, seventy fungal cultures obtained from sporocarps, and ninety-three isolates obtained from decayed wood samples (Table 2.3).

PCR amplification of extracted DNA: Typically, DNA was diluted 1/20 in TE buffer and 10 μl of diluted DNA was used as template in a 50 μl PCR reaction. The ribosomal DNA internal transcribed spacer (rDNA ITS) 1 and 2, including 5.8S and a part of 18S and 28S regions of nuclear rDNA, were amplified using the primer pair ITS1-F/ITS4. The forward primer, specific for fungi, ITS1-F (CTT GGT CAT TTA GAG GAA GTA A) (Gardes and Bruns 1993) was combined with a universal reverse primer ITS4 (TCC TCC GCT TAT TGA TAT GC) (White *et al.* 1990). Each PCR reaction contained 1 x polymerase buffer (Fisher Biotec), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 μM of each primer, and 0.08 U TTH+ polymerase (Fisher Biotech). Non-acetylated BSA (Bovine Serum Albumin, Fisher Biotec) was added at a final concentration of 0.2 mg/ml to reduce enzyme inhibition by plant or humic substance (Kreader 1996). Positive and negative controls were included in each set of PCR reactions. Amplification parameters were an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 7 minutes on a PTC 225 Peltier Thermal Cycler.

Electrophoresis: the amplified product was analysed by horizontal electrophoresis at 4 volts/cm, for 30 minutes in 2.5% agarose gel in 1 x TAE buffer. A 100 bp (base pair) DNA ladder (Fisher Biotec), was used for size estimation. The gel was stained in 1 μg ml^{-1} ethidium bromide by shaking slowly for 15 minutes. DNA was visualised under UV light and photographed with a Kodak EDAS290 camera, using Kodak IDLE version 3.5.

The proportion of DNA samples that did not amplify was 13.7% of the total 274 DNA amplifications attempted (86.3% were successfully amplified (Table 2.3)). The success rate of obtaining PCR amplification was greater for cultures (91.4 – 95.7%) than for sporocarps (68.5%).

Before sequencing, 90-100 μl of PCR product was purified using a MO BIO Laboratories, Inc. UltraClean™ PCR Clean-up Kit to remove all reaction components such as primers, enzyme, salt and dNTPs, then precipitated to concentrate the DNA according to instructions in the kit.

DNA sequencing and final ethanol precipitation were carried out according to the instructions provided with the Beckman Coulter GenomeLab Dye Terminator Cycle Sequencing with Quick Start Kit with the following modifications. Between 3 and 5 μl of DNA template were used in a 10 μl sequencing reaction with 3.2 pmol of primer and 2 μl of DTCS Quick Start Master Mix. During ethanol precipitation, 0.25 μl of 20 mg/ml of glycogen, 2 μl of 3 M Sodium Acetate and 2 μl of 100 mM Na_2-EDTA were added to each reaction. Sequences were determined on a Beckman Coulter CEQ™ 8000 Genetic Analysis System. Forward sequences were obtained for all sporocarp tissues and isolates, with reverse sequences only when additional information was required.

A total of 229 successful PCR products were sequenced. 86% of these products were successfully sequenced whilst 14 % failed to sequence or poorly sequenced (for more details, see Table 2.3). The success rate for obtaining a product and clean readable sequence was greater for cultures (88 – 94.4%) than for sporocarps (75%).

DNA sequences were edited using Chromas Lite version 2.0, 2004 (www.technelysium.com.au). Sequences of isolates or sporocarps from the same morphospecies were aligned in ClustalW (Thompson *et al.* 1994) and visually compared for similarity. Once a consensus sequence was obtained for each morphospecies, BLAST (Basic Local Alignment Search Tool) (Altschul *et al.* 1997) searches of public databases were carried out using BioManager (ANGIS 2005) or searches of private databases (ENSIS, University of Tasmania e.g. Potter (2005, unpublished data) sequences, Hopkins (2006) sequences) were conducted using FASTA program, version 3.4 (Pearson and Lipman 1988).

Table 2.3: Extraction, amplification and sequencing results for DNA from sporocarps, fungal cultures obtained from sporocarps, and isolates obtained from decayed wood.

Type of sample	Total number of samples from which DNA was extracted	PCR amplification of extracted DNA		Sequencing of PCR product		
				No success		
		No success	Success	Failed	Poor	Success
Sporocarp	111	(35) 31.5%	(76) 68.5%	(10) 13.1%	(9) 11.9%	(57) 75.0%
Fungal culture from sporocarp	70	(6) 8.6%	(64) 91.4%	(3) 4.2%	(5) 7.8%	(56) 88.0%
Isolates from decayed wood	93	(4) 4.3%	(89) 95.7%	(2) 2.2%	(3) 3.4%	(84) 94.4%
Total	274	(45) 13.7%	(229) 86.3%	(15) 6.5%	(17) 7.5%	(197) 86.0%

2.4. Development of a molecular technique to identify fungi from decayed wood

2.4.1. Direct identification

Twenty-six samples (Appendix 2.3) of rotten wood were taken from 19 decayed logs at six sites (Table 2.4.1) and characterised by Yee (2005). Two to seven subsamples were taken from each rot sample for DNA extraction with a total of 80 DNA samples (Table 2.4.1).

Wood sampling from decayed wood was carried out as described by Jasalavich *et al.* (2000) and was modified as follows: the decayed wood was sampled by drilling through wood surfaces. Precautions taken during drilling of the wood blocks to prevent cross-contamination of samples were similar to Jasalavich *et al.* (2000). However, instead of swabbing the worktable surface and gloves with 70% ethanol in between samples, the old sample was cleaned away and after putting on new gloves, a clean piece of tissue paper was placed on the worktable for the processing of each new sample. The same rechargeable cordless drill with a moulded housing was used for all samples but the 1/8 inch in diameter drill bit was inserted through a core of Whatman filter paper (9.0 cm diameter) and both the drill bit and filter paper were changed to the new ones for each time of sampling. The filter paper was positioned so as to cover the chuck and prevent sawdust from entering it. Each wood block was drilled through from 2 to 7 points (Table 2.4.1). Drilled material (100-200 μl) was transferred to a 1.5 ml eppendorf tube and ground further with a micropestle under liquid nitrogen. Then, 300-500 μl extraction buffer (Raeder & Broda 1985) was added and DNA extraction proceeded as described above for fungal sporocarps and cultures. PCR was carried out as above, using three different dilutions of the DNA extract (1/10, 1/20 and 1/50 in TE buffer).

Following amplification, PCR product was purified, precipitated, concentrated and sequenced as described previously.

All of the 80 extracted DNA samples were subjected to PCR with primers ITS1-F and ITS4. However, only eighteen DNA samples were successfully amplified. Sixty-two samples gave very faint or no visible PCR product after electrophoresis. Of those 18 successfully amplified DNA samples, seven showed multiple PCR products indicating that there would be more than one fungal species on those samples. Therefore only eleven samples were directly sequenced. One of those 11 samples returned no readable sequence results, and two of the 11 samples gave poor quality sequences. The remaining eight samples were successfully sequenced.

Table 2.4.1: The wood ID and sampling point.

Site[1]	Log code[2]	Wood ID[3]	Sample no.[4]
E	EDL1	(1) EDL1.1	S1
			S2
			S3
			S4
			S5
		(2) EDL1.6	S6
			S7
			S8
			S9
	EDS1	(3) EDS1.1	S10
			S11
		(4) EDS1.5	S12
			S13
			S14
			S15
			S16
	EDS2	(5) EDS2.5	S17
			S18
		(6) ERDS2.3	S19
			S20
			S21
			S22
			S23
			S24
	EDL2	(7) EDL2.1	S25
			S26
			S27
			S28
			S29
			S30
			S31
		(8) ERDL2.1	S32
			S33
			S34
		(9) EDL2.6	S35
			S36
	EDL3	(10) EDL3.3	S37
			S38
	ERDS3	(11) ERDS3.1	S39
			S40

[1]: Site name, referring to table 2.2.1, sites E, S, H and W were in Logging Regenerated Forest, sites M and WR were in Mature-unlogged Forest; [2]: The first letter denotes site (e.g. site E, excerpt ER = E, SW = S, MR = M and WR = site WR), the second letter "D" = diameter, the third letter "L" =large log or "S" = small log, the number denotes refers to the log sampled; [3]: The number after dot point denotes the sampling point on the disc cut from a log; [4]: number of drilled points or samples for each decayed wood sample.

Table 2.4.1 continued: The wood ID and sampling point.

Site[1]	Log code[2]	Wood ID[3]	Sample no.[4]
S (S)	SWDL2	(12) SWDL2.5	S41
			S42
	SWDS2	(13) SWDS2.1	S43
			S44
			S45
			S46
	SWDS3	(14) SWDS3 3	S47
			S48
			S49
			S50
			S51
			S52
		(15) SWDS3.32	S53
			S54
H	HDL3	(16) HL3.5	S55
			S56
	HDS2	(17) HC2.7	S57
			S58
			S59
M	MRDL2	(18) MRDL2.6	S60
			S61
	MRDS1	(19) MRDS1.4	S62
			S63
WR	WRDL2	(20) WRDL2.5	S64
			S65
	WRDS2	(21) WRDS2.3	S66
			S67
			S68
			S69
W	WRQ1	(22) WRQ1.14	S70
		(23) WRQ1.3b	S71
			S72
			S73
	SWQ1	(24) SWQ1.4	S74
			S75
	SWQ2	(25) SWQ2	S76
			S77
			S78
	SWQ3	(26) SWQ3 13	S79
			S80

[1]. Site name, referring to table 2.2.1, sites E, S, H and W were in Logging Regenerated Forest, sites M and WR were in Mature-unlogged Forest; [2]: The first letter denotes site (e.g. site E, excerpt ER = E, SW = S, MR = M and WR = site WR), the second letter "D" = diameter, the third letter "L" =large log or "S" = small log, the number denotes refers to the log sampled; [3]: The number after dot point denotes the sampling point on the disc cut from a log; [4]: number of drilled points or samples for each decayed wood sample.

2.4.2. DNA purification using polyvinylpolypyrrolidone

Further purification of DNA extracted from wood samples was carried out using polyvinylpolypyrrolidone (PVPP) chromatography: For those samples which gave faint or no PCR product a PVPP column method was used to further purify the DNA (Langrell 2005) before amplifying. Briefly, a total of 300 μl of sterile 10% PVPP solution was added to an empty spin column in a sterile 1.5 ml microcentrifuge tube. The tube was then centrifuged at 14,000 x g for one minute. The tube was emptied and centrifugation repeated to dry the PVPP matrix. The spin column was placed in a new 1.5 ml eppendorf tube. A solution of 100 μl of extracted DNA, diluted 1/10 in TE buffer was added to the spin column and centrifuged at 14,000 x g for one minute. The spin column was discarded and the purified DNA was stored at -20 °C.

Following amplification, a PCR product was purified, precipitated, concentrated and sequenced as described previously.

As a result, of the 61 DNA samples which underwent purification, 40 samples still gave no PCR product, nine initially gave a visible PCR product but failed to do so when the PCR was repeated, therefore did not provide sufficient template for sequencing. Twelve samples were amplified, but four samples showed multiple products after electrophoresis. Consequently, eight samples were sequenced. Five samples did not sequence, one sample gave a poor result and only two samples were successfully sequenced.

2.4.3. Cloning of PCR products

After amplification, seven PCR products obtained from decayed wood samples gave multiple bands on agarose gels, these seven PCR products were cloned using a pGEM^{R}-T (Promega) cloning kit.

The method followed the manufacturer's protocol. Before ligation, PCR products from wood samples were purified using the MO BIO Laboratories, Inc. UltraClean^{TM} PCR Clean-up Kit.

Ligation and transformation using pGEM^R-T vector were accomplished following the kit instructions. Each 10 μl ligation reaction contained 5 μl 2 X Rapid ligation buffer, 1 μl pGEM^R-T vector, 1 μl T4 DNA ligase and 3 μl PCR template. Positive, background and transformation controls were also performed. Reaction mix was centrifuged down at 14,000 rpm for 10 seconds, and was incubated overnight at 4 °C.

In order to obtain high transformation efficiency, JM109 High Efficiency Competent Cells provided by Promega were used to conduct experiment. 2 μl ligation reaction was transferred into each sterilised 1.5 ml eppendorf tube on ice, 2 μl uncut plasmid was transferred into another tube which was used to determine the transformation efficiency of the competent cells. 40 μl of thawed JM109 high efficiency competent cells were transferred into each of the above tube (80 μl cells for determination of transformation efficiency). The tubes were gently flicked to mix and placed on ice for 20 minutes. The cells were heat-shocked for 50 seconds in a water bath at exactly 42 °C then immediately returned to ice for 2 minutes. 950 μl room temperature SOC medium (in 100 ml, 2 g Bacto-tryptone, 0.5 g Bacto-yeast extract, 1 ml of 1M NaCl, 0.25 ml of 1 M KCl, 1 ml of 2 MMg^{2+} stock, 1 ml of 2 M glucose) was added to the tubes containing cells transformed with ligation reactions and 900 μl to the tube containing cells transformed with uncut plasmid. The tubes then were incubated for 1.5 hours at 37 °C with shaking (150 rpm). 100 μl of each transformation culture was placed onto duplicate antibiotic plates (LB plates with ampicillin/IPTG/X-Gal (10 g agar, 10 g Bacto^R-tryptone, 5 g Bacto^R-yeast extract, 5 g NaCl, 1000 ml water and 100 μg/ml ampicillin to make LB plates, 100 μl of 100 mM IPTG and 20 μl of 50 mg/ml X-Gal spread over the surface of the LB-ampicillin plate, allowing to absorb for 30 minutes at 37 °C prior to use)). For transformation control, 100 μl of a dilution 1:10 with SOC medium was transferred onto antibiotic plates. The plates were incubated overnight at 37 °C then placed at 10 °C to facilitate blue/white screening.

Individual white colonies (transformed colony) were transferred onto fresh LB/Amp/IPTG/X-Gal plate without cross contamination from other colonies, incubated overnight at 37 °C then stored at 4 °C.

A fast DNA extract was prepared from transformed colonies by lifting bacterial growth from the surface of medium with a sterile pipette tip and transferring it to a tube containing 50 μl TE buffer. Tubes were vortexed to disrupt bacterial cells then centrifuged to precipitate cell debris and the supernatant used for PCR with the ITS1-F/ITS4 primers. Products were electrophoresed to demonstrate successful amplification before screening by PCR-RFLP (PCR-Restriction fragment length polymorphism). The restriction enzymes *Hinf* I and *Taq* I were used separately. The clones were firstly screened for sequence variation by PCR-RFLP using *Hinf* I restriction enzymes. For those clones of which their DNAs were not cut by *Hinf* I restriction enzyme, they were secondly screened by PCR-RFLP using *Taq* I restriction enzyme. Each 10 μl reaction contained 1 U *Hinf* I or *Taq* I (Promega), the appropriate buffer as supplied by the manufacturer and 5 μl PCR product without prior purification. The reaction mix was incubated in thermocycler at 37 °C (*Hinf* I) or 65 °C (*Taq* I) for 4 to 6 hours.

The digested products were separated by electrophoresis in a 25 cm gel tray in 2.5% Hi-Resolution agarose (Progen Industries Limited) at 100 volts for 5 hours with 3 marker lanes in each row.

Clones from each of the wood samples were grouped according to their PCR-RFLP profiles and representatives of each PCR-RFLP type were sequenced. PCR was repeated as above and sequencing as described previously.

DNA sequences were edited using Chromas Lite version 2.0, 2004 (www.technelysium.com.au), then used to search public databases (Genbank, EMBL, DDBJ) with BLAST (Altschul *et al.* 1997) and private databases (CSIRO Ensis FFP, including all of the above sporocarp sequences, fungal culture sequences obtained from sporocarps and isolate sequences obtained from decayed logs) with FASTA, version 3.4 (Pearson and Lipman 1988).

2.5. Analysing database search result

Five levels of sequence matching were accepted as indicative of identification to different taxonomic levels.

1A) Sequences were considered likely to belong to the same species obtained from BLAST search or private searches if there were less than 1-2% variation (or over 98% match) between them over at least 400 bp and no other species had higher than 95% similarity. Consideration must also be given to the current status of taxonomic knowledge of the species and genus and the availability of ITS sequences from other species in that genus.

1B) If only one species had greater than 95% similarity to the query sequence and all others less than 90%, the unknown fungus is regarded as close to that species, e.g. *Ganoderma* aff. *lucidum*.

2) If several species had greater than 95% similarity to the query sequence, a phylogenetic analysis was performed to determine the most likely placement of the unknown fungus. Sequences of study and reference fungi were aligned and dendrograms were created using Clustalw (Thompson *et al.* 1994) and DNAml of the Phylip package (ANGIS 2005; Felsenstein 1989) and viewed in TreeView version 1.6.6 (Page 2001).

3) If several species from a single genus had greater than 90% match to the query sequence, the unknown species was considered likely to be another species belong to that genus.

4) If several species from more than one genus and the same family had sequence similarity higher than 80%, the unknown species was considered likely to belong to that family.

5) In cases where no reasonable matches are found to the ITS sequence, the search results provide a list of species with high similarity to the 5.8S region, which is highly conserved and is located between ITS1 and ITS2. This is usually sufficient only to discriminate a higher level of phyla of fungi – Ascomycota, Basidiomycota and Zygomycota.

3. Results

3.1. Obtaining a reference collection of sequences to allow the molecular identification of wood decay fungi direct from wood samples

Sequences were derived from 57 of the 111 sporocarps and 56 of the 70 isolates obtained from those sporocarps. Sequences were obtained from both cultures and sporocarps for 23 specimens. For the remaining analyses, sequences were either obtained from the sporocarp or the isolate, not both. For 21 sporocarps no sequence information was obtained from either sporocarp tissue or corresponding cultures.

3.1.1. Molecular identifications support morphological identification

Thirty sequences obtained directly from sporocarp tissue supported the original morphological identifications attributed to the sporocarps analysed:

- ✓ Seven of the 30 identifications agreed to species level and sixteen were supported at genus level (Table 3.1.1.1). The molecular identifications of cultures isolated from 13 sporocarps that were not analysed directly from their tissue also agreed with the species morphological identifications attributed to their 'parent' sporocarps (Fig.3.1.1).
- ✓ One sporocarp (E7587) was morphologically identified as *Ganoderma* sp. but the molecular identification refined this identification to *G. applanatum* (Fig.A2.4.11).
- ✓ Two sporocarps (E7546 and E7551) morphologically identified as *Schizophyllum commune* had identical sequences and DNA analysis confirmed this species.
- ✓ The morphological identifications of 16 sporocarps were confirmed only to genus level. Of these one sporocarp (16722A) was identified by phylogenetic analysis as *Ganoderma* sp.1, closest to *Ganoderma* sp. JM95/5 and JM95/6 (Genbank accession numbers: AF255117 and AF255121 respectively). Six sporocarps were identified as *Ganoderma* sp.3, closest to *Ganoderma* sp. BJ-7, BJ-8 which were oil palm *Ganoderma* isolates obtained from *Elaeis guineensis* (Utomo *et al.* 2005) (Genbank accession numbers: AY220539 and AY220540 respectively). For analysis of the *Ganoderma* specimens, see Fig.A2.4.11.

- ✓ Four sporocarps identified morphologically to species level could only confidently be identified by DNA analysis to family level (according to the criteria set out in section 2.5) though the closest BLAST search results agreed with the morphological identification to genus level. Although the query sequences were of high quality, most of them only matched the Genbank reference sequences in the 5.8S and part of the ITS1 (e.g. E7562 and E7568) or ITS2 (e.g. E7543) regions. E7537 had an incomplete sequence (409bp) yet a 300bp portion matched with accessed sequences at the ITS1 and a part of the 5.8S region. For sporocarps E7537, E7543, E7562 and E7568 no previous ITS sequence information was available on Genbank or private databases for the species identifications attributed to the sporocarps i.e. *Xerula radicata, Mycena austrocapillara, Psathyrella echinospora* and *Hypoxylon rubiginosum.* An additional sporocarp E7550 was morphologically identified as a species of Xylariaceae and this was confirmed by molecular analysis but not further resolved.
- ✓ Two sporocarps (E7560 and E7590) were identified by sequence analysis only to phylum level. The sequence for E7560 had high similarity with accessed sequences from Genbank in the 5.8S region only, and E7590 gave a high match (97%) with database sequences but different families were represented in the highest matches.

DNA sequence matches for twenty-three of the 56 fungal isolates (41.07%) supported the morphological identification of the sporocarps from which the fungal isolates had been cultured (Table 3.1.1.2).

Ten of the 23 matches supported the original morphological identification of the sporocarp at a species level with a high percent similarity in the ITS1 and ITS2 regions to sequences in Genbank.

- ✓ Four isolates (P0840, P0904, P0909 and P0910) obtained from *Trametes* sporocarps were identified by their ITS sequences to species level.
- ✓ Two isolates (P0842 and P0846) had identical sequences and were identified as *Schizophyllum commune*, the same species as the sporocarps from which the cultures were derived (E7546 and E7551).
- ✓ Four isolates (P0837, P0866, P0893 and P0913) matched or improved upon the original sporocarp identifications of E7539, E7582, E7616 and E7514.

Four of the 23 isolates (P0918, P0844, P0878, and P0892) had high similarity matches to a sequence of the same species as the sporocarp from which the culture was derived. However, according to criteria in section 2.5 confidence in identification could only be supported to genus level.

Another seven isolates (P0835, P0843, P0849, P0851, P0852, P0887 and P0917) were also identified to genus level, in agreement with sporocarp morphological identifications.

The sequence for isolate P0875, derived from a sporocarp that had been identified as *Fistulina hepatica,* matched this species in the 5.8S region with no significant matches in the ITS regions and no closer matching species (Table 3.1.1.2). Therefore identification was only considered reliable to the level of order Aphyllophorales.

One isolate (P0922) obtained from a sporocarp identified morphologically as *Trametes ochracea* was grouped with *T. versicolor* in a phylogenetic analysis (Fig.A2.4.8).

Table 3.1.1.1: Molecular identification from sporocarp tissues supported morphological identification of these sporocarps.

Herbarium code	Morphological identification of sporocarp	Sequence available[1]	Mol ID		Closest Blast search results for sporocarp tissue		Length[6]	Match (%)
			Level of confidence	Name	Access code	Species		
16452D1	*Ganoderma* sp.	N/A	2	*Ganoderma* sp.3	AY220542	*Ganoderma* sp. BJ-7	560/640	99
16453C1	*Ganoderma* sp.	N/A	2	*Ganoderma* sp.3	AY220543	*Ganoderma* sp. BJ-7	543/583	99
16453C2	*Ganoderma* sp.	N/A	2	*Ganoderma* sp.3	AY220542	*Ganoderma* sp. BJ-7	560/640	99
16453C3	*Ganoderma* sp.	N/A	2	*Ganoderma* sp.3	AY220542	*Ganoderma* sp. BJ-7	560/640	99
16722A	*Ganoderma* sp.	N/A	2	*Ganoderma* sp.1	AF255122	*Ganoderma* sp. JM 95/5	640/640	98
167311B	*Ganoderma* sp.	N/A	2	*Ganoderma* sp.3	AY220542	*Ganoderma* sp. BJ-7	560/635	99
16732C	*Ganoderma* sp.	N/A	2	*Ganoderma* sp.3	AY220542	*Ganoderma* sp. BJ-7	561/640	99
E7536	*Pycnoporus coccineus*	Yes	3	*Pycnoporus* sp.	AF363768	*Pycnoporus cinnabarinus*	492/689	92
E7537	*Xerula radicata*	No	4	Tricholomataceae	AY026919	*Xerula furfuracea*	293/409	90
E7539	*Pleurotopsis longinqua*	Yes	1B	*P.* aff. *longinqua*	AF012195	*Pleurotopsis longinqua*	402/402	99
E7540	*Armillaria hinnulea*	Yes	3	*Armillaria* sp.	AF394918	*Armillaria hinnulea*	390/400	91
E7542	*Ganoderma australe*	Yes	1A	*G. australe*	AY884180	*Ganoderma australe*	630/657	98
E7543	*Mycena austrocapillara*	No	4	Tricholomataceae	AY787675	*Mycena* sp.	245/704	94
E7544	*Trametes versicolor*	Yes	2	*T.* aff. *versicolor*	AY968080	*Trametes versicolor*	534/540	98
E7546	*Schizophyllum commune*	Yes	1A	*S. commune*	AF280758	*Schizophyllum commune*	540/540	98
E7547	*Gymnopilus pampeanus*[2]	Yes	2	*Gymnopilus* sp.2	AY280997	*Gymnopilus pampeanus*[2]	420/420	100
E7550	*Xylariaceae* sp.	N/A	4	Xylariaceae	AY315407	*Xylariaceae* sp.	289/480	93
E7551	*Schizophyllum commune*	Yes	1A	*S. commune*	AF280758	*Schizophyllum commune*	479/630	99
E7553	*Rhodocollybia butyracea*	Yes	1B	*R* aff *butyracea*	AY313290	*Rhodocollybia butyracea*	511/670	96
E7555	*Cortinarius* sp.	N/A	3	*Cortinarius* sp.	DQ097877	*Cortinarius alboviolaceus*	609/610	94
E7559	*Amauroderma rude*	Yes	3	*Amauroderma* sp.	AJ627583	*Amauroderma subresinosum*[3]	619/630	92
E7560	*Phellinus* sp.	N/A	5	Homobasidiomycetes	AY558647	*Phellinus senex*	239/690	96

Table 3.1.1.1 (continued).

Herbarium code	Morphological identification of sporocarp	Sequence available[1]	Mol ID		Closest Blast search results for sporocarp tissue		Length[6]	Match (%)
			Level of confidence[6]	Name	Access code	Species		
E7562	*Psathyrella echinospora*	No	4	Psathyrellaceae	AF082578	*Psathyrella spadiceogrisea*	146/486	92
E7565	*Armillaria* sp.	N/A	3	*Armillaria* sp.	AY213561	*Armillaria calvescens*	115/760	93
E7568	*Hypoxylon rubiginosum*	No	4	Xylariaceae	AY303629	*Hypoxylon diatrypeoides*	217/480	92
E7582	*Gymnopilus allantopus*	Yes	3	*Gymnopilus* sp.	AF501542	*Gymnopilus allantopus*	236/600	97
E7587	*Ganoderma* sp.	N/A	2	*G.* aff. *applanatum*[4]	AJ608709	*Ganoderma applanatum*[4]		
E7590	*Trametes versicolor*	Yes	5	Homobasidiomycetes	AB158314	*Trametes ochracea*	559/600	97
E7595	*Stereum ostrea*	Yes	3	*Stereum* sp.	DQ000294	*Stereum annosum*[5]	445/588	95
E7615	*Steccherinum litschaueri*	Yes	3	*Steccherinum* sp.	AY781278	*Steccherinum litschaueri*	545/565	95

[1]N/A: not applicable; [2]*Gymnopilus pampeanus* currently accepteted as *Pseudogymnopilus pampeanus*; [3]*Amauroderma subresinosum* currently accepteted as *Ganoderma subresinosum*; [4]*G. applanatum* currently accepteted as *G. australe*; [5]*Stereum annosum* currently accepted as *Xylobolus annosus*; [6]Length of matching region/length of submitted region [6]see section 2.5 in Methods

Table 3.1.1.2: Molecular identification from fungal cultures obtained from sporocarps supported morphological identification of these sporocarps (sorted by isolate code).

Herbarium code	Morphological identification of sporocarp	Sequence available[1]	Mol ID		Isolate Code	Closest Blast search result for fungal culture		Length[6]	Match (%)
			Level of confidence[7]	Name		Access code	Species		
E7536	*Pycnoporus coccineus*	Yes	2	*Pycnoporus* sp.	P0835	AF363768	*Pycnoporus cinnabarinus*	460/598	99
E7539	*Pleurotopsis longinqua*	Yes	1A	*P.* aff. *longinqua*	P0837	AF012195	*Pleurotopsis longinqua*	621/660	97
E7544	*Trametes versicolor*	Yes	2	*T.* aff. *versicolor*	P0840	AY968080	*Trametes versicolor*	608/626	98
E7546	*Schizophyllum commune*	Yes	1A	*S. commune*	P0842	AF350925	*Schizophyllum commune*	461/480	98
E7547	*Gymnopilus pampeanus*[2]	Yes	2	*Gymnopilus* sp.2	P0843	AY280988	*Gymnopilus junonius*	475/480	96
E7549	*Hypholoma fasciculare*	Yes	3	*Hypholoma* sp.	P0844	AY354216	*Hypholoma fasciculare*	556/574	97
E7551	*Schizophyllum commune*	Yes	1A	*S. commune*	P0846	AF280758	*Schizophyllum commune*	616/623	98
E7559	*Amauroderma rude*	Yes	3	*Amauroderma* sp.	P0849	AJ627583	*Amauroderma subresinosum*[3]	576/586	92
E7563	*Pholiota highlandensis*	Yes	3	*Pholiota* sp.	P0851	AY251301	*Pholiota carbonaria*[4]	205/572	95
E7564	*Stereum ostrea*	Yes	3	*Stereum* sp.	P0852	DQ000294	*Stereum annosum*[5]	496/650	94
E7582	*Gymnopilus allantopus*	Yes	2	*G.* aff. *allantopus*	P0866	AY219590	*Gymnopilus allantopus*	470/480	99
E7591	*Fistulina hepatica*	Yes	5	Aphyllophorales	P0875	AY571038	*Fistulina hepatica*	170/400	98
E7595	*Stereum ostrea*	Yes	3	*Stereum* sp.	P0878	AF082856	*Stereum ostrea*	461/494	97
E7611	*Xerula australis*	Yes	3	*Xerula* sp.	P0887	AF321480	*Xerula furfuracea*	410/540	93
E7615	*Steccherinum litschaueri*	Yes	3	*Steccherinum* sp.	P0892	AY781278	*Steccherinum litschaueri*	307/519	95
E7616	*Phlebia* sp.	N/A	2	*P.* aff. *radiata*	P0893	DQ056859	*Phlebia radiata*	537/540	97
E7505	*Trametes hirsuta*	Yes	2	*T.* aff. *hirsuta*	P0904	AY972129	*Trametes hirsute*	440/480	100
E7510	*Trametes hirsuta*	Yes	2	*T.* aff. *hirsuta*	P0909	AF516556	*Trametes hirsuta*	453/480	96
E7511	*Trametes hirsuta*	Yes	2	*T.* aff. *hirsuta*	P0910	AF516556	*Trametes hirsuta*	456/480	96
E7514	*Anthracophyllum* sp.	N/A	1A	*A. lateritium*	P0913	DQ444309	*Anthracophyllum lateritium*	600733	98
E7518	*Stereum* sp.	N/A	3	*Stereum* sp.	P0917	DQ000294	*Stereum annosum*[5]	396/540	95
E7519	*Panellus stypticus*	Yes	3	*Panellus* sp.	P0918	AF289063	*Panellus stypticus*	162/360	99
E7528	*Trametes ochracea*	Yes	2	*T.* aff. *versicolor*	P0922	AB158314	*Trametes ochracea*	413/420	100

[1]N/A: not applicable, [2]*Gymnopilus pampeanus* currently accepted as *Pseudogymnopilus pampeanus*; [3]*Amauroderma subresinosum* currently accepted as *Ganoderma subresinosum* [4]*Pholiota carbonaria* currently accepted as *Pholiota highlandensis*; [5]*Stereum annosum* currently accepted as *Xylobolus annosus*; [6]Length of matching region/length of submitted region.
[7]see section 2.5 in Methods

The molecular analyses for 10 sporocarps matched the morphological identifications of these 10 sporocarps and the molecular identifications of the 10 fungal cultures isolated from these sporocarps (Fig.3.1.1, Table 3.1.1.1 and Table 3.1.1.2).

- ✓ Four of these 10 matches were identified to species level by molecular analyses (E7539, E7544, E7546 and E7551).
- ✓ The ITS sequence of sporocarp E7547 was identical to that from the fungal culture derived from it (P0843) and resulted in identification as *Gymnopilus* sp.2 but not further resolved. (Fig.A2.4.2).
- ✓ The remaining five matches could only be identified to genus level by sequence similarity.

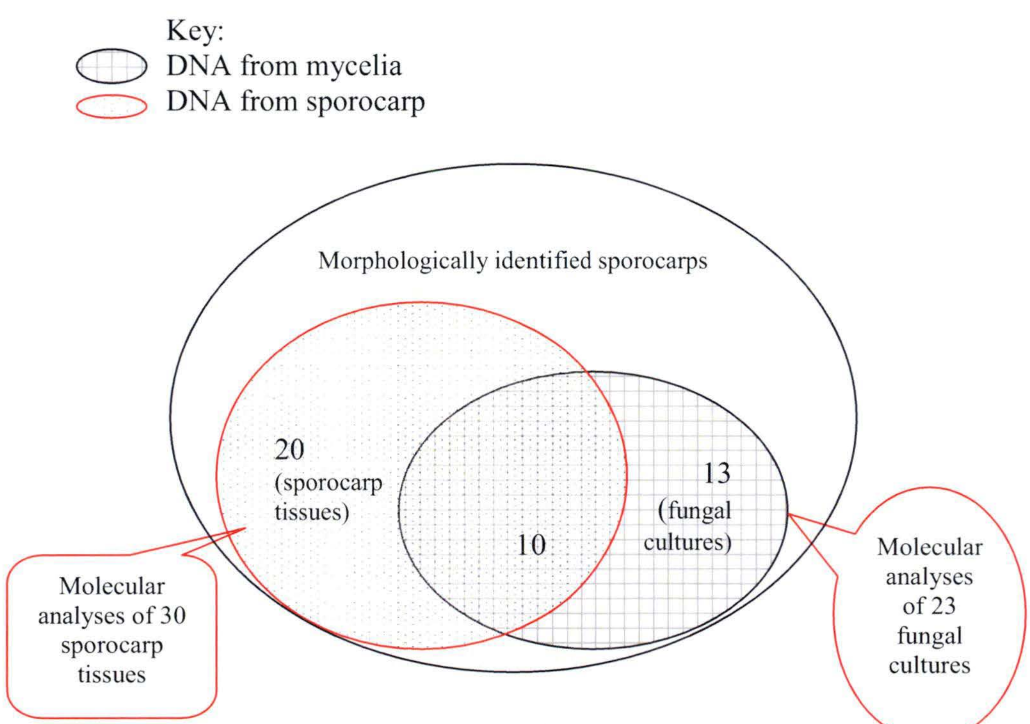


Figure 3.1.1: Molecular identification agrees (at different taxonomic levels) with morphological identification for 43 sporocarps.

3.1.2. Molecular identifications do not support morphological identifications

Nighteen morphologically identified sporocarps fall into this category. Eleven sporocarps and twelve isolates cultured from the 19 sporocarps were sequenced successfully. There were only 4 cases in which sequences were obtained from both the sporocarps and their corresponding isolates. For the remaining analyses, sequences were either obtained from the sporocarp or the isolate, not both.

The molecular analysis of sporocarp tissues and corresponding fungal isolates did not support the sporocarp morphological identifications.

- ✓ Molecular identification did not match morphological identification for eleven of the 57 sporocarps (Table 3.1.2.1).
- ✓ Only two sequences (E7557 and E7597) gave a species level identification but these species identifications were different to morphological identification of the sporocarp from which they were derived e.g. the molecular identification of E7597 as the gilled species *Lentinellus pulvinulus* (Fig.A2.4.7) was different from morphological identification of the sporocarp as *Postia* sp. which has pores.
- ✓ Sporocarps E7601 and E7602 were identified to a genus that conflicted with their morphological identification.
- ✓ Sporocarps E7554 and E7567 were identified to a family level as query sequences only matched at 5.8S and ITS2 regions with databased sequences. Molecular confirmation of the identification of sporocarps E7601 and E7554 was compromised by the lack of available sequences for *Ryvardenia* sp. and *Fomes hemitephrus*.
- ✓ Five sporocarps were identified to class or phylum level. Two sporocarp samples (E7577 and E7603) produced poor quality sequences, possibly due to contamination. Two others (E7541 and E7613) gave high quality sequences, but searches only returned matches in the 5.8S region. The sequence of one sporocarp (E7545) was of high quality and had a moderate (92%) match to other fungi in Genbank but it could only be confidently identified as Heterobasidiomycetidae since it had moderate similarity to several species from different families.

Table 3.1.2.1: Molecular identification from sporocarp tissues versus morphological identification of sporocarps.

Herbarium code	Morphological identification of sporocarp	Sequence available[1]	Mol ID		Closest Blast search result for sporocarp tissue		Length[3]	Match (%)
			Level of confidence[4]	Name	Access code	Species		
E7541	*Pulvinula archeri*	Yes	5	Ascomycota	AF351581	*Mycorrhizal otidean*	236/579	97
E7545	Phlebioid sp.	N/A	5	Heterobasidiomycetidae	AF291272	*Eichleriella deglubens*	514/572	92
E7554	*Fomes hemitephrus*	No	4	Ganodermataceae	AJ627583	*Amauroderma subresinosum*[2]	376/540	92
E7557	*Tyromyces* sp.	N/A	1A	*T. cucumeris*	DQ426529	*Thanatephorus cucumeris*	443/600	99
E7567	*Xylobolus* sp.	N/A	4	Fomitopsidaceae	AJ006667	*Postia sericeomollis*	231/540	91
E7577	Phlebioid sp.	N/A	5	Homobasidiomycetes	AY049142	*Grifola sordulenta*	181/480	88
E7597	*Postia* sp.	N/A	2	*Lentinellus* aff. *pulvinulus*	AY513138	*Lentinellus pulvinulus*	466/480	99
E7601	*Ryvardenia* sp.	No	3	*Postia* sp.	AJ006666	*Postia balsamea*	377/660	91
E7602	*Athelia* sp.	N/A	3	*Mycena* sp.	AF335445	*Mycena* sp.	441/739	95
E7603	*Anthracophyllum* sp.	N/A	5	Homobasidiomycetes	AY281005	*Gymnopilus robustus*	176/653	97
E7613	*Aleurodiscus* sp.	N/A	5	Ascomycota	AY348598	*Lambertella* sp.	245/720	92

[1]N/A: not applicable; [2]*Amauroderma subresinosum* currently accepted as *Ganoderma subresinosum*; [3]Length of matching region/length of submitted region
[4]see section 2.5 in Methods

For twelve of the 56 closest matches with sequences from fungal isolates there was no support for the morphological identification of the sporocarps from which the fungal isolates were derived (Table 3.1.2.2).

Molecular identification was possible to species level for three fungal isolates (P0860, P0867 and P0897). For analysis of P0897, see Fig.A2.4.4.

- ✓ For another three isolates, molecular identification could not be verified below genus level. Fungal culture P0884 was identified as *Phanerochaete* sp.1 (Fig.A2.4.6), most likely because no ITS sequence for *Ryvardenia* was on available databases. *Phanerochaete* is a resupinate genus and clearly different from the *Ryvardenia* sporocarp from which P0844 had been isolated. The other three isolates identified to genus level were P0850 (morphologically identified as *Phellinus* and identified as *Ganoderma* by molecular analyses), P0874 (morphologically identified as *Trametes versicolor* and identified as *Psilocybe* sp. by molecular analyses) and P0900 (morphologically identified as *Phlebia rufa* and identified as *Stereum* sp. by molecular analyses).
- ✓ The two isolates obtained from two different sporocarps of *Antrodiella zonata* (P0915 and P0919) had identical ITS sequences. Lack of known sequence information for *A. zonata* and *Fomes hemitephrus* (P0847) compromised molecular identification of these species, which were only verified to family level using ITS sequence data.
- ✓ Two cultures (P0885 and P0853) gave identification to order and class level.

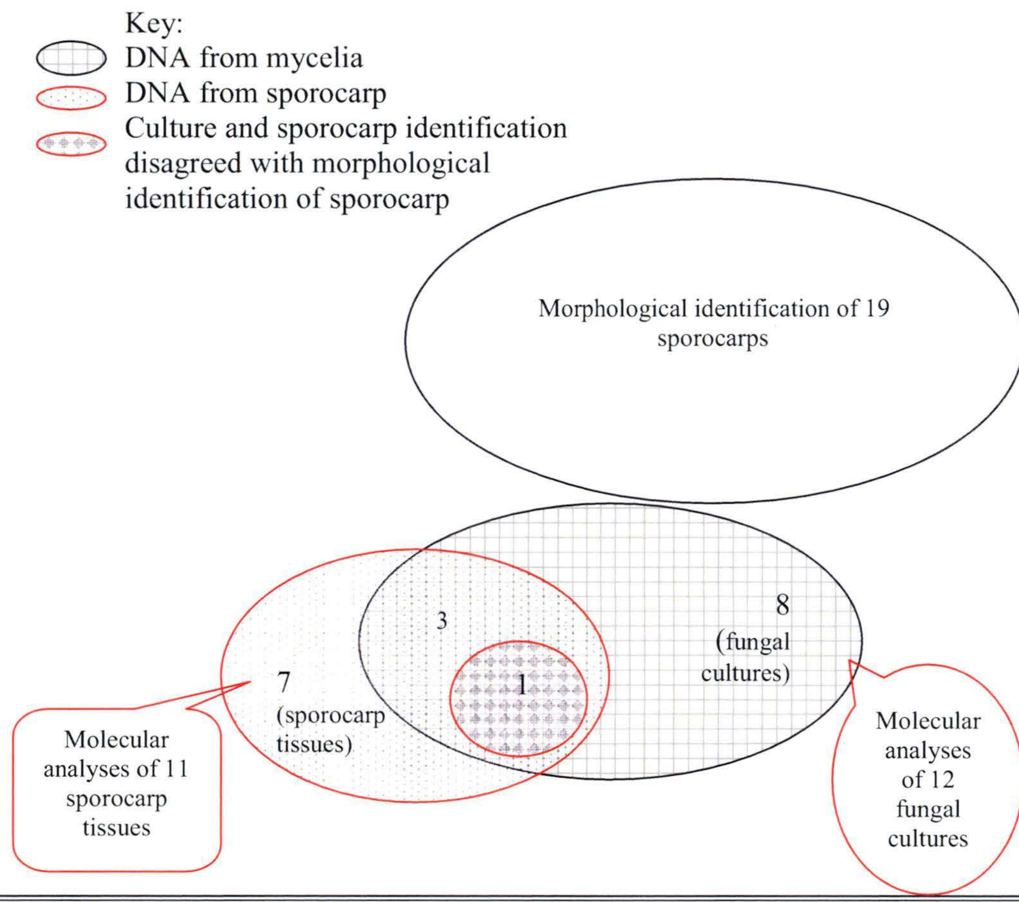
DNA sequences for three sporocarps (E7602, E7567 and E7554) were identical to sequences from their corresponding fungal isolates (P0885, P0853 and P0847) however their morphological identifications were verified only to family, order or class (Fig.3.1.2, Table 3.1.2.1 and Table 3.1.2.2). For E7601 the molecular analysis of sporocarp tissue and its corresponding isolate (P0884) differed from each other and from the morphological identification of the sporocarp (Table 3.1.2.1 and Table 3.1.2.2).

Table 3.1.2.2: Molecular identification from fungal cultures versus morphological identification of sporocarps.

Herbarium code	Morphological identification of sporocarp	Sequence available[1]	Mol ID		Isolate code	Closest Blast search result for fungal culture		Length[4]	Match (%)
			Level of confidence[5]	Name		Access code	Species		
E7554	*Fomes hemitephrus*	No	4	Ganodermataceae	P0847	AJ627583	*Amauroderma subresinosum*[3]	496/650	94
E7560	*Phellinus* sp.	N/A	3	*Ganoderma* sp.	P0850	AJ608709	*Ganoderma* sp.	375/480	99
E7567	*Xylobolus* sp.	N/A	5	Homobasidiomycetes	P0853	AY672926	*Postia sericeomollis*	316/614	89
E7576	*Gloeoporus taxicola*[2]	Yes	1A	*Umbelopsis ramanniana*	P0860	AB193539	*Umbelopsis ramanniana*	467/480	98
E7583	*Schizophyllum* sp.	N/A	1A	*Gymnopilus allantopus*	P0867	AF501542	*Gymnopilus allantopus*	658/670	100
E7590	*Trametes versicolor*	Yes	3	*Psilocybe* sp.	P0874	PCO519795	*Psilocybe coprophila*	361/765	92
E7601	*Ryvardenia* sp.	No	2	*Phanerochaete* sp.1	P0884	AY781266	*Phanerochaete sordida*	426/600	96
E7602	*Athelia* sp.	N/A	5	Agaricales	P0885	AF335445	*Mycena* sp.	376/600	93
E7558	*Polyporus* sp.	N/A	2	*Simplicillium* aff. *lamellicola*	P0897	AB214656	*Simplicillium lamellicola*	592/596	98
E7501	*Phlebia rufa*	Yes	3	*Stereum* sp.	P0900	AY781272	*Stereum sanguinolentum*	555/620	92
E7516	*Antrodiella zonata*	No	4	Polyporaceae	P0915	DQ056858	*Cerrena unicolor*	541/540	91
E7520	*Antrodiella zonata*	No	4	Polyporaceae	P0919	AY456192	*Cerrena unicolor*	447/480	90

[1]N/A: not applicable; [2]*Gloeoporus taxicola* currently accepted as *Meruliopsis taxicola*; [3]*Amauroderma subresinosum* currently accepted as *Ganoderma subresinosum* [4]Length of matching region/length of submitted region [5]see section 2.5 in Methods

Figure 3.1.2: Molecular identification does not agree with the morphological identification for 19 sporocarps.



3.1.3. Molecular identifications in the absence of morphological identifications

Sequences were derived i) directly from 15 of the 27 morphologically unidentified sporocarps (Table 3.1.3.1) and ii) 21 isolates obtained from these 27 unidentified sporocarps, mainly resupinate fungi (Table 3.1.3.2). Sequences were obtained, in 9 instances, from both the unidentified sporocarp and its corresponding isolate (Fig.3.1.3). The remaining sequences were therefore obtained from 6 unidentified sporocarps and 12 isolates derived from different unidentified sporocarps.

✓ Sequences from three sporocarps (E7552, E7574 and E7575) provided reliable identification at species level (for analysis of E7552, see Fig.A2.4.3).

- ✓ Five sequences provided identification to genus level; sequences from two sporocarps (E7592 and E7593) were identical to each other and determined as *Phanerochaete* sp.2 (Fig.A2.4.6); one sequence matched *Psilocybe* sp. but was probably a contaminant as the sequence was derived from a resupinate sporocarp (E7579). E7589 and E7596 were determined to be *Gymnopilus* sp. and *Hypocrea* sp. respectively.
- ✓ Three sequences (E7573, E7586 and E7604) gave identifications to family level.
- ✓ The remaining four sequences only matched to class or order level.

For the isolates derived from unidentified sporocarps that were mainly resupinate fungi:

- ✓ Six were identified to species level (for analyses of P0859 and P0916, see Fig.A2.4.5 and Fig.A2.4.10 respectively). Two of these (P0895 and P0905) were contaminants.
- ✓ Another seven isolates were identified to genus level (Table 3.1.3.2). The phylogenetic analysis of P0876 and P0877 showed that they were identical to each other and were identified as *Phanerochaete* sp.2 (Fig.A2.4.6).
- ✓ One isolate (P0882) was identified to family level.
- ✓ Four isolates could only be identified to order; three to Aphyllophorales (P0902, P0920 and P0923,) and one (P0869) to Saccharomycetales (most likely a contaminant).
- ✓ The remaining three isolates (P0879, P0881 and P0886) were identified to class, phylum.

In the six of the 9 cases where DNA sequences were obtained from a sporocarp and its isolate, the sequences from sporocarp and isolate were identical. However one pair (E7579 and P0863; Table 3.1.3.1 and Table 3.1.3.2) were identified as *Psilocybe* sp., contrary to the sporocarp morphology.

In the other three cases (E7586, E7596 and E7599; Table 3.1.3.1 and Table 3.1.3.2, Fig.3.1.3), molecular identification of the isolates, though only to class or phylum level, indicated that a contaminant fungal species had been isolated.

Figure 3.1.3: The sporocarp tissue and/or culture mycelium from 27 sporocarps that had not been morphologically identified, were identified by DNA sequence analysis.

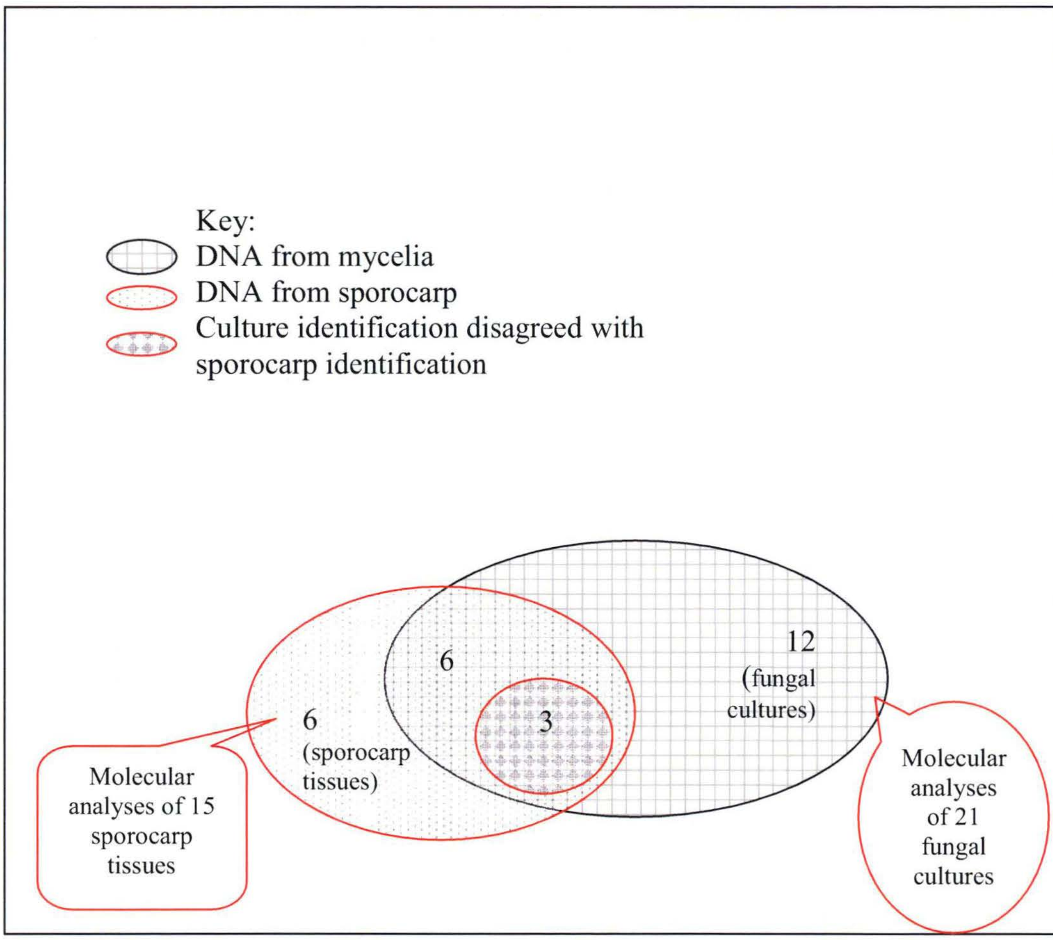


Table 3.1.3.1: Molecular identification of sporocarp tissue in the absence of morphological identification.

Herbarium code	Morphological identification of sporocarp	Sequence available[1]	Mol ID		Closest Blast search result for sporocarp tissue		Length[5]	Match (%)
			Level of confidence[6]	Name	Access code	Species		
E 7552	Unidentified	N/A	2	*Hypocrea* aff. *sulphurea*	DQ000634	*Hypocrea sulphurea*	591/615	99
E 7569	Unidentified	N/A	5	Homobasidiomycetes	AY 805630	*Gloeoporus taxicola*[2]	183/726	99
E 7573	Unidentified	N/A	4	Cortinariaceae	AF389162	*Dermocybe cardinalis*	155/629	92
E 7574	Unidentified	N/A	1A	*Bisporella citrina*	AF335454	*Bisporella citrina*	549/550	99
E 7575	Unidentified	N/A	1B	*Phlebia* aff. *radiata*	AY854087	*Phlebia radiata*	626/628	96
E 7579	Resupinate	N/A	3	*Psilocybe* sp.	PCO519795	*Psilocybe coprophila*	512/644	95
E 7586	Resupinate, white	N/A	4	Atheliaceae	AY751564	*Atheliaceae* sp.	208/540	94
E 7589	Unidentified	N/A	3	*Gymnopilus* sp.	AF501542	*Gymnopilus allantopus*	360/480	97
E 7592	Resupinate	N/A	2	*Phanerochaete* sp.2	AY219376	*Phanerochaete sordida*	520/540	97
E 7593	Resupinate	N/A	2	*Phanerochaete* sp.2	AY219376	*Phanerochaete sordida*	527/540	97
E 7596	Resupinate, yellow	N/A	3	*Hypocrea* sp.	DQ000634	*Hypocrea sulphurea*	394/420	97
E 7598	Unidentified	N/A	5	Agaricales	AF361318	*Collybia cirrhata*[3]	215/654	91
E 7599	Resupinate	N/A	5	Homobasidiomycetes	AJ420946	*Gloeophyllum sepiarium*	169/650	99
E 7604	Unidentified	N/A	4	Clavicipitaceae	BTU35287	*Beauveria tenella*[4]	171/463	90
E 7605	Resupinate, yellow	N/A	5	Homobasidiomycetes	AY219354	*Phanerochaete carnosa*	350/540	93

[1]N/A: not applicable; [2]*Gloeoporus taxicola* currently accepted as *Meruliopsis* taxicola; [3]*Collybia cirrhata* currently accepted as *Collybia cookei*; [4]*Beauveria tenella* currently accepted as *B. brongniartii*; [5]Length of matching region/length of submitted region [6]see section 2.5 in Method

Table 3.1.3.2: Molecular identification of fungal culture obtained from sporocarp tissue in the absence of morphological identification.

Herbarium code	Morphological identification of sporocarp	Sequence available[1]	Mol ID		Isolate code	Closest Blast search result for fungal culture		Length[3]	Match (%)
			Level of confidence[4]	Name		Access code	Species		
E 7575	Unidentified	N/A	2	*Phlebia* aff. *radiata*	P0859	AY854087	*Phlebia radiata*	417/420	97
E 7578	Resupinate	N/A	1A	*Coniophora* aff. *puteana*	P0861	CPU344110	*Coniophora puteana*	621/660	98
E 7579	Resupinate	N/A	3	*Psilocybe* sp.	P0863	PCO519795	*Psilocybe coprophila*	510/576	94
E 7585	Unidentified	N/A	3	*Ceriporiopsis* sp.	P0868	AY781250	*Ceriporiopsis* sp.	571/621	95
E 7586	Resupinate, white	N/A	5	Saccharomycetales	P0869	AY493435	*Stephanoascus ciferrii*	187/510	94
E 7589	Unidentified	N/A	1A	*Gymnopilus* aff. *allantopus*	P0872	AF501542	*Gymnopilus allantopus*	647/680	100
E 7592	Resupinate	N/A	2	*Phanerochaete* sp.2	P0876	AF475150	*Phanerochaete sordida*	620/620	97
E 7593	Resupinate	N/A	2	*Phanerochaete* sp.2	P0877	AF475150	*Phanerochaete sordida*	605/617	96
E 7596	Resupinate, yellow	N/A	5	Zygomycete	P0879	AY627838	Root associated fungus	580/600	99
E 7599	Resupinate	N/A	5	Hymenomycetes	P0881	AY583324	*Marchandiomyces aurantiacus*	183/540	98
E 7600	Resupinate, small white spots	N/A	4	Apiosporaceae	P0882	AY425967	*Arthrinium* sp.	436/600	94
E 7605	Resupinate, yellow	N/A	5	Homobasidiomycetes	P0886	AY219354	*Phanerochaete carnosa*	350/540	93
E 7614	Resupinate	N/A	3	*Peniophora* sp.	P0889	AY787677	*Peniophora cinerea*	557/600	98
E 7618	Resupinate, yellow-white, thick	N/A	1A	*Umbelopsis isabellina*	P0895	AY354280	*Umbelopsis isabellina*	519/517	97
E7503	Unidentified	N/A	5	Aphyllophorales	P0902	AY787678	*Peniophora limitata*	189/420	96
E7506	Unidentified	N/A	1A	*U. ramanniana*	P0905	AB193539	*Umbelopsis ramanniana*	454/480	98
E7508	Unidentified	N/A	2	*Peniophora* sp.	P0907	AF210828	*Peniophora aurantiaca*	468/480	98
E7517	Unidentified	N/A	2	*Hypholoma* aff. *fasciculare*	P0916	AY354216	*Hypholoma fasciculare*	500/540	97
E7526	Unidentified	N/A	5	Aphyllophorales	P0920	AJ627584	*Ganoderma philippii*	242/480	97
E7529	Unidentified	N/A	5	Aphyllophorales	P0923	AJ627584	*Ganoderma philippii*	242/480	97
E7534	Unidentified	N/A	3	*Stereum* sp.	P0928	DQ000294	*Stereum annosum*[2]	333/480	98

[1]N/A: not applicable; [2]*Stereum annosum* currently accepted as *Xylobolus annosus*; [3]Length of matching region/length of submitted region. [4]see section 2.5 in Methods

3.1.4. Morphological identifications in the absence of molecular identifications

Only nine cultural isolations were obtained from the 23 sporocarps listed in the Table 3.1.4; eight *Ganoderma* specimens originating from the Northern Territory had been preserved in alcohol therefore cultural isolation was impossible; isolations from another six sporocarps were not successful. The majority of these 23 sporocarps had been morphologically identified. DNA analyses were not successful for any of the 23 sporocarps or for the nine isolations.

The DNA extractions from the five specimens of *Polyporus* sp. and their respective isolates did not amplify with the primers used here, or if amplifiable gave poor sequencing results. DNA extracted from six of the eight sporocarps of *Ganoderma* did not amplify, and the PCR products of the other two sporocarps of this *Ganoderma* species produced unreadable sequencing results.

Table 3.1.4: Unsuccessful molecular analyses with sporocarp and fungal culture.

Herbarium code	Morphological identification of sporocarp	Molecular attempt with sporocarp	Isolate	Molecular attempt with isolate
15948B1	*Ganoderma* sp.	DNA not amplified	Isolation not possible	
15948B2	*Ganoderma* sp.	DNA not amplified	Isolation not possible	
15948B3	*Ganoderma* sp.	DNA not amplified	Isolation not possible	
16313A1	*Ganoderma* sp.	DNA not amplified	Isolation not possible	
16313A2	*Ganoderma* sp.	DNA not amplified	Isolation not possible	
16313A3	*Ganoderma* sp.	DNA not amplified	Isolation not possible	
16452D2	*Ganoderma* sp.	Poor sequences	Isolation not possible	
16452D3	*Ganoderma* sp.	Failed sequencing	Isolation not possible	
E 7535	*Ryvardenia* sp.	DNA not amplified	Successful	Failed sequencing
E 7538	*Polyporus* sp.	Poor sequence	Successful	DNA not amplified
E 7548	*Polyporus* sp.	DNA not amplified	Successful	DNA not amplified
E 7561	*Hymenochaete* sp.	Failed sequencing	Unsuccessful isolate	
E 7566	Resupinate	Poor sequences	Unsuccessful isolate	
E 7580	*Polyporus* sp.	DNA not amplified	Successful	DNA not amplified
E 7581	*Hypholoma fasciculare*	DNA not amplified	Successful	Failed sequencing
E 7584	*Inonotus* sp.	Failed sequencing	Unsuccessful isolate	
E 7588	Mycoacia	Failed sequencing	Successful	Poor sequences
E 7594	Agaric, small delicate	Failed sequencing	Unsuccessful isolate	
E 7612	*Polyporus* sp.	DNA not amplified	Successful	DNA not amplified
E 7617	*Polyporus* sp.	DNA not amplified	Successful	DNA not amplified
E 7619	*Artomyces* sp.	Failed sequencing	Unsuccessful isolate	
E 7620	*Mycena* sp.	Poor sequences	Unsuccessful isolate	
E 7621	*Dermocybe* sp.	DNA not amplified	Successful	Poor sequence

3.1.5. Molecular identification of isolates from decayed logs

3.1.5.1. Molecular identification agreed with a previous identification from cultural morphology

In this group, identification by molecular techniques supported the previous morphological identification of five cultures although the levels of support varied (Table 3.1.5.1).

- ✓ To species level with one isolate, LF2 was identified as *Trametes versicolor* (see Fig.A2.4.8).
- ✓ The molecular analysis of isolate LF249 gave a more precise identification than culture morphology. It was identified as *Armillaria hinnulea* (see Fig.A2.4.14).
- ✓ Two isolates previously identified as *Ganoderma* sp.1 (LF569 and LF400) both gave sequence matches to *G. applanatum* (GenBank accession number: AJ608709); according to the criteria used only LF569 was accepted as *G. applanatum*, the other isolate (LF400) was classified as *Ganoderma* sp.2 (see Fig.A2.4.11).

Table 3.1.5.1: Molecular identification from isolates obtained from decayed logs supported cultural identification of those isolates.

Isolate Code	Cultural identification of isolate	Sequence available[1]	Mol ID		Closest Blast search result for isolate		Length[3]	Match (%)
			Level of confidence[4]	Name	Access code	Species		
LF2	*Trametes versicolor*	Yes	2	*Trametes* aff. *versicolor*	AY309017	*Trametes versicolor*	441/540	99
LF4	*Stereum ostrea*	Yes	3	*Stereum* sp.	AY089730	*Stereum sanguinolentum*	376/480	96
LF249	*Armillaria* sp.	N/A	2	*Armillaria* aff. *hinnulea*	AF394918	*Armillaria hinnulea*	704/740	96
LF400	*Ganoderma* sp.1	N/A	2	*Ganoderma* sp.2	AJ608709	*Ganoderma applanatum*[2]	505/540	97
LF569	*Ganoderma* sp.1	N/A	2	*Ganoderma* aff. *applanatum*	AJ608709	*Ganoderma applanatum*[2]	538/540	99

[1]N/A: not applicable; [2]*Ganoderma applanatum* currently accepted as *Ganoderma australe*; [3]Length of matching region/length of submitted region [4]see section 2.5 in Methods

3.1.5.2. Molecular identifications do not support previous cultural identifications

Nineteen isolates had been identified in previous studies (Hopkins 2006) by their characteristic morphology in culture or the appearance of the mycelium in the wood (e.g. *Polyporus mylittae* was named by Yuan on the basis of isolation from a sclerotium, embedded within the rotten wood, that was similar to those formed by *P. mylittae*. However, several other species of *Laccocephalum* also form sclerotia, and sclerotium characters are insufficient to distinguish among species).

Eleven isolates that had been identified, as *P. mylittae* (Table 3.1.5.2), since renamed *Laccocephalum mylittae*. The closest BLAST match results for those isolates were *Diplomitoporus lindbladii* (currently accepted as *Poria lindbladii*) and *Postia subcaesia* (currently accepted as *Tyromyces subcaesius*), though no sequences of *L. mylittae* were available for comparison. A herbarium specimen of *L. mylittae* was obtained (collector number: E5568, Perth, Australia) and the ITS sequenced, but this sequence did not match that of any of the 11 isolates which were therefore accepted as belonging to a different species in the Polyporaceae.

Four isolates (LF204, LF173, LF3 and LF12) were identified to species level. LF 173 is probably a contaminant. LF12 previously identified as *Phellinus wahlbergii* had a 100% match to the widespread wood rotting resupinate species *Coniophora puteana.*

One isolate (LF5) was identified to genus level; LF5 previously identified as *Xylobulus illudens* did not have a corresponding sequence available in the databases.

Two isolates (LF59 and LF 67) previously identified as *P. wahlbergii* and *Postia caesia* were both identified by molecular analyses as the same species in the family Xylariaceae (for phylogenetic analysis of LF59 and LF67, see Fig.A2.4.12).

One specimen (LF68) previously identified as *Fomes hemitephrus* also did not have any corresponding sequence in the databases and was classed by molecular analysis as in the Aphyllophorales.

Table 3.1.5.2: Molecular identification of isolate obtained from decayed logs differed from cultural identification of those isolates.

Isolate Code	Cultural identification of isolate	Sequence available[1]	Mol ID		Closest Blast search result for isolate		Length[5]	Match (%)
			Level of confidence[6]	Name	Access code	Species		
LF68	*Fomes hemitephrus*	No	5	Aphyllophorales	AJ627584	*Ganoderma philippii*	242/480	97
LF173	*Mycena* sp.1	N/A	1A	*Mortierella verticillata*	AY997063	*Mortierella verticillata*	566/640	99
LF3	*Phellinus robustus*	Yes	1A	*Fomitiporia australiensis*	AY624995	*Fomitiporia australiensis*	369/420	99
LF12	*Phellinus wahlbergii*	Yes	1A	*Coniophora puteana*	CPU344110	*Coniophora puteana*	479/480	100
LF59	*Phellinus wahlbergii*	Yes	2	*Xylariaceae* sp.1	AY843084	*Xylariaceae* sp.	417/420	98
LF207	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	DLI6682	*Diplomitoporus lindbladii*[3]	530/630	93
LF246	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	DLI6682	*Diplomitoporus lindbladii*[3]	613/620	92
LF247	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	AY599577	*Postia subcaesia*[4]	395/650	89
LF252	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	AJ006682	*Diplomitoporus lindbladii*[3]	530/540	92
LF361	*Polyporus mylittae*[2]	Yes	5	Aphyllophorales	AF454429	*Auriscalpium villipes*	170/640	98
LF508	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	AJ006682	*Diplomitoporus lindbladii*[3]	380/540	93
LF519	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	AJ006682	*Diplomitoporus lindbladii*[3]	529/540	92
LF521	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	AJ006682	*Diplomitoporus lindbladii*[3]	591/726	91
LF527	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	AJ006682	*Diplomitoporus lindbladii*[3]	532/540	92
LF539	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	AJ006682	*Diplomitoporus lindbladii*[3]	518/540	93
LF646	*Polyporus mylittae*[2]	Yes	4	Polyporaceae	AJ006682	*Diplomitoporus lindbladii*[3]	512/540	92
LF204	*Poria* sp.2 (pink)	N/A	1B	*Gymnopilus* aff. *allantopus*	AF501542	*Gymnopilus allantopus*	655/680	97
LF67	*Postia caesia*	No	2	*Xylariaceae* sp.1	AY315402	*Xylariaceae* sp.	405/420	99
LF5	*Xylobulus illudens*	No	3	*Stereum* sp.	AY805632	*Stereum sanguinolentum*	343/420	93

[1]N/A: not applicable; [2]*Polyporus mylittae* currently accepted *Laccocephalum mylittae*; [3]*Diplomitoporus lindbladii* currently accepted *Poria indbladii*; [4]*Postia subcaesia* currently accepted *Tyromyces subcaesius*; [5]Length of matching region/length of submitted region [6]see section 2.5 in Methods

3.1.5.3. Molecular identification of morphologically grouped, unidentified isolates.

Twenty nine isolates had been grouped in previous studies (Hopkins 2006) according to cultural morphology but the isolates had not been identified.

- ✓ Six isolates had been grouped together as *Basidio*-sp.3. Four isolates of this group were classified on the basis of their DNA sequences as Homobasidiomycetes (Table 3.1.5.3). Sequences from these four isolates had high similarity (94-96%) to each other, but not to any database sequences except in the 5.8S region. The other two isolates (LF81 and LF75) were identified as Aphyllophorales or Pezizomycotina respectively. LF81 was 98.5% similar to isolate LF646, 99.8% to isolate LF539, and 99.2% similar to LF63. Isolates LF646 and LF 539 had been morphologically identified as *P. mylittae.*
- ✓ Four isolates, grouped as *Basidio*-sp.6 were identified to genus level, *Antrodia.* The high sequence similarity in this group (0.7-1.5% variation) verified the morphological grouping of the isolates.
- ✓ Two isolates grouped as *Basidio*-sp.8 were identified as *Zygomycota* and are therefore not Basidiomycota but contaminants or misidentifications.
- ✓ Two isolates grouped as "brown colony fungi" (LF383 and LF477) were identified as Polyporaceae. The closest match for both these isolates was *P. subcaesia,* currently accepted as *T. subcaesius* in the Polyporaceae. One representative isolate (LF613) of the so-called "Paper-S fungus" group was also identified as Polyporaceae. These three sequences had similarities of 88-95%.
- ✓ Single isolates from each of the groups "Duckling fungus" and "Whitening radiating fungus" were identified as Aphyllophorales with 81.3% sequence similarity between them. The closest matches to these two isolates were two different species of *Phlebia.* One representative isolate of "Whitening cottony fungus" group gave a very poor sequence, and could only be determined as belonging to the kingdom Fungi.
- ✓ Three isolates from different morphological groupings (*Basidiomycete* sp.5, sp.13 and sp.14) were identified as belonging to the genus *Xylaria.* LF46 was determined to species level; LF118 and LF89 were identical and grouped in phylogenetic analysis as *Xylariaceae* sp.1 (see Fig.A2.4.12).

Table 3.1.5.3: Molecular identification of grouped isolates.

Isolate Code	Cultural identification of isolate	Sequence available[1]	Mol ID		Closest Blast search result for isolate		Length[6]	Match (%)
			Level of confidence[7]	Name	Access code	Species		
LF61	*Basidio*-sp.1	N/A	4	Fomitopsidaceae	AJ 006666	*Postia balsamea*	334/689	90
LF62	*Basidio*-sp.1	N/A	5	Agaricales	AY445119	*Agaricus* sp.	162/420	97
LF47	*Basidio*-sp.10	N/A	4	Meripilaceae	ASI416068	*Antrodia sinuosa*	539/600	87
LF46	*Basidio*-sp.13	N/A	2	*Xylaria* aff. *castorea*	AF163030	*Xylaria castorea*	411/420	98
LF89	*Basidio*-sp.14	N/A	2	*Xylariaceae* sp.1	AY315402	*Xylariaceae* sp.	527/540	98
LF102	*Basidio*-sp.15	N/A	3	*Ceriporiopsis* sp.	AY781250	*Ceriporiopsis* sp.	509/540	95
LF96	*Basidio*-sp.18	N/A	5	Aphyllophorales	AY354219	*Phlebia tremellosa*[5]	250/540	96
LF97	*Basidio*-sp.19	N/A	5	Homobasidiomycetes	DQ056859	*Phlebia radiata*	143/557	87
LF63	*Basidio*-sp.2	N/A	5	Aphyllophorales	DLI6682	*Diplomitoporus lindbladii*[2]	518/540	92
LF75	*Basidio*-sp.3	N/A	5	Pezizomycotina	AY781221	*Dactylaria* sp.	444/480	97
LF77	*Basidio*-sp.3	N/A	5	Homobasidiomycetes	DQ056859	*Phlebia radiata*	142/300	88
LF78	*Basidio*-sp.3	N/A	5	Homobasidiomycetes	DQ056859	*Phlebia radiata*	143/346	87
LF79	*Basidio*-sp.3	N/A	5	Homobasidiomycetes	DQ056859	*Phlebia radiata*	142/360	88
LF80	*Basidio*-sp.3	N/A	5	Homobasidiomycetes	DQ056859	*Phlebia radiata*	143/420	87
LF81	*Basidio*-sp.3	N/A	5	Aphyllophorales	DLI6682	*Diplomitoporus lindbladii*[2]	529/540	93
LF93	*Basidio*-sp.4	N/A	3	*Postia* sp.	AY218416	*Oligoporus rennyi*[3]	322/670	93
LF118	*Basidio*-sp.5	N/A	2	*Xylariaceae* sp.1	AY315402	*Xylariaceae* sp.	534/540	98
LF141	*Basidio*-sp.6	N/A	3	*Antrodia* sp.	AXA415569	*Antrodia xantha*	376/540	93
LF142	*Basidio*-sp.6	N/A	3	*Antrodia* sp.	AXA415569	*Antrodia xantha*	386/540	92
LF143	*Basidio*-sp.6	N/A	3	*Antrodia* sp.	AXA6681	*Antrodia xantha*	391/540	92
LF146	*Basidio*-sp.6	N/A	3	*Antrodia* sp.	AXA415569	*Antrodia xantha*	393/540	92
LF35	*Basidio*-sp.8	N/A	5	Zygomycota	AY805542	*Umbelopsis isabellina*	159/420	94
LF48	*Basidio*-sp.8	N/A	5	Zygomycota	AY997097	*Umbelopsis ramanniana*	159/420	95
LF383	Brown colony fungus	N/A	4	Polyporaceae	AY599577	*Postia subcaesia*[4]	545/660	88
LF477	Brown colony fungus	N/A	4	Polyporaceae	AY599577	*Postia subcaesia*[4]	391/480	86
LF653	Duckling fungus	N/A	5	Aphyllophorales	AY354219	*Phlebia tremellosa*[5]	373/540	91
LF613	Paper-S fungus	N/A	4	Polyporaceae	AY599576	*Postia subcaesia*[4]	393/540	89
LF281	Whitening cottony fungus	N/A	5	Fungi	AY805596	*Verticillium* sp.	164/480	88
LF637	Whitening radiating fungus	N/A	5	Aphyllophorales	AY590781	*Phlebia radiata*	316/480	88

[1]N/A: not applicable; [2]*Diplomitoporus lindbladii*, current name = *Poria lindbladii*; [3]*Oligoporus rennyi* current name = *Postia rennyi*, [4]*Postia subcaesia*, current name = *Tyromyces subcaesius*; [5]*Phlebia tremellosa* current name = *Merulius tremellosus*; [6]Length of matching region/length of submitted region. [7]see section 2.5 in Methods.

3.1.5.4. Molecular identification in the absence of cultural identification or grouping

Thirty-one unidentified isolates were identified by molecular techniques in this study but the levels of confidence in identification varied (Table 3.1.5.4).

Two isolates (LF155 and LF157) were identified to species level as *Sistotrema brinkmannii* and *G. applanatum* respectively (for analysis of LF157, see Fig.A2.4.11).

Ten isolates were identified to genus level. LF154 and LF583 were identified as different species of *Hypocrea* respectively (see Fig.A2.4.15 for analysis of LF583); LF216 was identified as *Penicillium* (Fig.A2.4.13); LF159 and LF352 were different species of *Ganoderma* (for analysis of LF159, see Fig.A2.4.11); LF192 and LF302 were identical to each other and identified as *Xylariaceae* sp.1 (Fig.A2.4.12); LF389 was identified as *Gymnopilus* sp.1 (Fig.A2.4.2); LF 495 was identical to P0916 and identified as *Hypholoma* sp. (Fig.A2.4.10) and LF 610 as *Pestalotiopsis* sp.

Four isolates were identified to family; six to order; two to subclass; six to phylum (4 Zygomycota and 2 Ascomycota); one, due to the poor quality of the sequence could only be verified as belonging to the kingdom Fungi.

Table 3.1.5.4: Molecular identification of unidentified isolates.

Isolate Code	Cultural identification of isolate	Sequence available[1]	Mol ID		Closest Blast search result for isolate		Length[6]	Match (%)
			Level of confidence[7]	Name	Access code	Species		
LF154	Unidentified isolate	N/A	3	*Hypocrea* sp.	DQ093709	*Hypocrea koningii*	481/480	98
LF155	Unidentified isolate	N/A	1A	*Sistotrema brinkmannii*	DQ093653	*Sistotrema brinkmannii*	459/480	98
LF156	Unidentified isolate	N/A	5	Agaricales	AY805621	*Pholiota spumosa*	274/480	97
LF157	Unidentified isolate	N/A	2	*Ganoderma* aff. *Applanatum*[2]	AF255158	*Ganoderma applanatum*[2]	421/480	99
LF158	Unidentified isolate	N/A	4	Hymenochaetaceae	AF255158	*Fomitiporia australiensis*	315/540	99
LF159	Unidentified isolate	N/A	2	*Ganoderma* sp.	AY593862	*Ganoderma fornicatum*	240/540	96
LF175	Unidentified isolate	N/A	5	Aphyllophorales	AF429421	*Hypochnicium albostramineum*	464/640	90
LF184	Unidentified isolate	N/A	4	Cortinariaceae	AJ585465	*Galerina calyptrata*	410/540	94
LF192	Unidentified isolate	N/A	2	*Xylariaceae* sp.1	AY315402	*Xylariaceae* sp.	504/540	96
LF196	Unidentified isolate	N/A	5	Zygomycota	AY997097	*Umbelopsis ramanniana*	158/660	95
LF200	Unidentified isolate	N/A	5	Zygomycota	AY997097	*Umbelopsis ramanniana*	158/640	95
LF208	Unidentified isolate	N/A	5	Saccharomycetales	AY344066	*Candida fragi*	167/510	96
LF216	Unidentified isolate	N/A	2	*Penicillium* sp.	AY373934	*Penicillium thomii*	560/560	97
LF234	Unidentified isolate	N/A	5	Homobasidiomycetes	AJ006671	*Spongipellis spumeus*	175/640	100
LF301	Unidentified isolate	N/A	5	Ascomycota	AY354276	*Ascomycete* sp.	438/580	97
LF302	Unidentified isolate	N/A	2	*Xylariaceae* sp.1	AY315402	*Xylariaceae* sp.	556/579	97
LF312	Unidentified isolate	N/A	5	Zygomycota	AY781213	*Zygomycete* sp.	136/835	92
LF352	Unidentified isolate	N/A	3	*Ganoderma* sp.	AJ627583	*Amauroderma subresinosum*[3]	369/540	91
LF356	Unidentified isolate	N/A	5	Ascomycota	AY618687	*Ascomycete* sp.	503/550	95
LF357	Unidentified isolate	N/A	5	Aphyllophorales	TCH6676	*Tyromyces chioneus*	123/540	95
LF358	Unidentified isolate	N/A	5	Aphyllophorales	TCH6676	*Tyromyces chioneus*	122/620	95
LF389	Unidentified isolate	N/A	2	*Gymnopilus* sp.1	AF501562	*Gymnopilus tyallus*	639/670	99
LF417	Unidentified isolate	N/A	5	Fungi	AF438566	*Boletus edulis*	35/660	97
LF495	Unidentified isolate	N/A	2	*Hypholoma* sp.	AF438590	*Hypholoma fasciculare*	359/540	96
LF579	Unidentified isolate	N/A	4	Fomitopsidaceae	AJ006666	*Postia balsamea*	390/600	90
LF583	Unidentified isolate	N/A	2	*Hypocrea* sp.	AY240841	*Trichoderma album*[4]	531/620	100
LF588	Unidentified isolate	N/A	5	Homobasidiomycetes	AF506379	*Boidinia propinqua*	299/600	91
LF593	Unidentified isolate	N/A	4	Fomitopsidaceae	AJ006666	*Postia balsamea*	392/540	90

Table 3.1.5.4 (continued).

Isolate Code	Cultural identification of isolate	Sequence available[1]	Mol ID		Closest Blast search result for isolate		Length[6]	Match (%)
			Level of confidence[7]	Name	Access code	Species		
LF610	Unidentified isolate	N/A	3	*Pestalotiopsis* sp.	DQ001003	*Pestalotiopsis microspora*	251/550	95
LF642	Unidentified isolate	N/A	5	Zygomycota	AY997097	*Umbelopsis ramanniana*	166/680	94
LF644	Unidentified isolate	N/A	5	Aphyllophorales	AJ006682	*Diplomitoporus lindbladii*[5]	530/540	92

[1]N/A: not applicable; [2]*Ganoderma applanatum* currently accepted as *Ganoderma australe*; [3]*Amauroderma subresinosum* currently accepted as *Ganoderma subresinosum*; [4]*Trichoderma album* currently accepted as *Hypocrea citrina*; [5]*Diplomitoporus lindbladii* currently accepted as *Poria indbladii*; [6]Length of matching region/length of submitted region [7]see section 2.5 in Methods

3.2. Identification of wood decay fungi direct from wood samples

Although DNA was extracted from 26 rotten wood samples PCR products were obtained from only 17 samples and PVPP purification was required for two samples before PCR was successful. Readable sequences were obtained from 10 PCR products (Table 3.2.1).

- ✓ Six of the 10 sequences were identified as Basidiomycota; three were classified to the order Aphyllophorales; two in the class Homobasidiomycetes; S71 was identified to genus level, *Ganoderma* sp.3 (Fig.A2.4.11). This species in phylogenetic analysis groups close to six sequences of *Ganoderma* specimens which were collected in Northern Territory and close to *Ganoderma* sp. BJ-7, BJ-8 and GB-1 which were oil palm *Ganoderma* isolates obtained from *Elaeis guineensis* (Utomo *et al.* 2005) (Fig.A2.4.11). Two of the Aphyllophorales (S53, S60) had 98.8-99.8% similarity to the cultures LF63, LF81, LF207, LF246, LF252, LF508, LF519, LF521, LF527, LF539, LF644 and LF646, many of which had been morphologically identified as *P. mylittae.*
- ✓ Another four sequences were determined as Ascomycota; three to phylum level and one (S46) to genus level.

Table 3.2.1: Molecular identification of fungi directly from decayed logs.

Wood ID	Sample no.	Mol ID		Blast code	Closest Blast search result for wood decay fungi[1]	Length[3]	Match (%)
		Level of acceptance[4]	Name				
ERDL2.1	S34	5	Homobasidiomycetes	AF377204	*Tricholoma* sp.*	178/790	93
MRDL2.6	S60	5	Aphyllophorales	AJ006682	*Diplomitoporus lindbladii*[1]	480/538	91
SWDL2.5	S41	5	Ascomycota	AJ635314	*Oidiodendron myxotrichoides**	54/955	94
SWDS2.1	S46	3	*Rhizoscyphus* sp.	AY112936	*Hymenoscyphus ericae*[2]	393/600	92
SWDS3.3	S52	5	Homobasidiomycetes	AF508346	*Bridgeoporus nobilissimus*	174/480	98
SWDS3.32	S53	5	Aphyllophorales	AJ006682	*Diplomitoporus lindbladii*[1]	483/480	92
WRDS2.3	S66	5	Ascomycota	AF486129	*Sporendocladia foliicola*	360/360	92
WRDS2.3	S69	5	Ascomycota	AF486129	*Sporendocladia foliicola*	352/360	91
WRQ1.14	S70	5	Aphyllophorales	AJ415551	*Fomitopsis rosea*	132/540	90
WRQ1.3b	S71	2	*Ganoderma* sp.3	AY220542	*Ganoderma* sp.	560/660	99

[1]*Diplomitoporus lindbladii* currently accepted as *Poria lindbladii*; [2]*Hymenoscyphus ericae* currently accepted as *Rhizoscyphus ericae*; * the identification of wood decayed fungi after PVPP cleaning up; [3]Length of matching region/length of submitted region [4]see section 2.5 in Methods

Seven PCR products from five rotted wood samples had multiple sequences. These products were cloned and clones screened by PCR-RFLP. Representatives from each of 24 PCR-RFLP groups were sequenced (Table 3.2.2). Three DNA samples S21, S23 and S24 originated from a single rotten wood sample (ERDS2.3). Sequence results from these three sub-samples were extremely diverse (Table 3.2.2) and accounted for over half of the 24 sequences. For the other four rotten wood samples only a single DNA extract per sample succeeded in PCR.

- ✓ Most of the identifications were to phylum or class because matches were only in the 5.8S region or matches were to several fungi from diverse groups. Eighteen cloned ITS sequences were derived from Ascomycota and five from Basidiomycota and one from Zygomycota.
- ✓ Two cloned sequences (S23d and S80) were identified to genus level. One (S80 *Mycena* sp.) was a potential wood rotter; S23d is more likely to be a fungal endophyte. S80 matched (82.3 to 93.4 % sequence similarity) with sporocarps E7543, E7602 and culture P0885 (obtained from sporocarp E7602).
- ✓ Only three cloned sequences (S24e, S40c and S62b) were identified to species level (see Fig.A2.4.13, Fig.A2.4.16 and Fig.A2.4.17) but none of these were considered as potential primary wood rotters.

Table 3.2.2: Molecular identification of wood decay fungi after cloning of PCR products.

Wood ID	Sample no.		Mol ID		Blast code	Closest Blast search result for wood decay fungi	Length[4]	Match (%)
			Level of acceptance	Name				
ERDS2.3	S21	(a)	5	Homobasidiomycetes	AJ006677	*Skeletocutis amorpha*	124/531	100
		(b)	5	Ascomycota	AY787735	*Ascomycete* sp.	483/570	97
		(c)	5	Pezizomycotina	AY805585	*Cadophora malorum*	250/600	97
	S23	(a)	5	Sordariomycetes	AY618685	*Sporothrix* sp.	179/600	89
		(b)	5	Sordariomycetes	AY786143	*Togninia minima*[1]	202/570	96
		(c)	5	Zygomycota	AY781213	*Zygomycete* sp.	447/630	96
		(d)	3	*Oidiodendron* sp.	AF062787	*Oidiodendron pilicola*	491/539	90
		(e)	5	Helotiales	AY606305	*Phialocephala dimorphospora*	430/576	89
	S24	(a)	5	Helotiales	AF486120	*Mycelium radicis-atrovirens*	482/590	88
		(b)	5	Ascomycota	AY805586	*Phialophora* sp.	380/580	91
		(c)	5	Ascomycota	AJ784399	*Oidiodendron setiferum*	257/720	89
		(d)	5	Pezizomycotina	AY781242	*Cadophora* sp.	425/538	89
		(e)	2	*Penicillium* aff. *spinulosum*	AY373933	*Penicillium spinulosum*	565/582	98
ERDS3.1	S40	(a)	5	Pezizomycotina	AY618685	*Sporothrix* sp.	92/620	90
		(b)	5	Pezizomycotina	AJ293884	*Ophiostoma grandicarpum*[2]	180/570	98
		(c)	2	*Oidiodendron* aff. *rhodogenum*	AF062803	*Oidiodendron rhodogenum*	506/560	99
		(d)	5	Ascomycota	AY618687	*Ascomycete* sp.	296/580	95
SWDS3.3	S51	(a)	5	Homobasidiomycetes	AY854085	*Grifola sordulenta*	173/590	98
		(b)	5	Homobasidiomycetes	AB084621	*Phlebia uda*[3]	172/556	99
		(c)	5	Pezizomycotina	AY112936	cf. *Hymenoscyphus* sp.	529/550	90

Table 3.2.2 (continued): Molecular identification of wood decay fungi after cloning of PCR products.

Wood ID	Sample no.	Mol ID		Blast code	Closest Blast search result for wood decay fungi	Length[4]	Match (%)
		Level of acceptance[5]	Name				
MRDS1.4	S62 (a)	5	Homobasidiomycetes	AF506468	*Scytinostroma ochroleucum*	407/720	95
	(b)	2	*Penicillium* aff. *citreonigrum*	AY373908	*Penicillium citreonigrum*	578/580	99
	(c)	4	Trichocomaceae	AY373864	*Aspergillus restrictus*	523/570	95
SWQ3.13	S80	3	*Mycena* sp.	AF335445	*Mycena* sp.	650/660	95

[1]*Togninia minima*, current name = *Phaeoacremonium aleophilum*; [2]*Ophiostoma grandicarpum*, current name = *Ceratocystis grandicarpa*; [3]*Phlebia uda* current name = *Mycoacia uda*; [4]Length of matching region/length of submitted region. [5]see section 2.5 in Methods

4. Discussion

This project has been valuable as a pilot study for understanding the problems involved in the detection of wood decay fungi direct from rotting wood samples. It has also added a number of new sequences of fungi associated with coarse woody debris and attached to named or tagged sporocarps and/or cultures that can be included in both Genbank and the Hobart Forest Health sequence databases. Only 11 sporocarps out of 96 were collected at Warra. Most came from northern Tasmania. Since the objective of the sporocarp collection was to establish a reference set for investigating wood decay fungi at Warra it may have been better to make more collections at this site rather than in different locations/soil and forest types and with different silvicultural histories. However most of the wood decay species collected are ubiquitous in Tasmania and found at Warra (Gates, personal communication) and the information from this thesis is still applicable to ecological studies of the fungal diversity in the wet sclerophyll forest of southern Tasmania at Warra. Ultimately such studies will inform industry on how best to manage the forest matrix for conservation values.

The strategy described in Fig.1.2.1 for establishing reference sequences for wood decay fungi (i.e. matching cultures to identified sporocarps) was successful albeit the total number of reference sequences determined was limited.

- Out of 111 sporocarps and 70 isolates obtained from them, sequencing definitively coupled only 10 pairs of sporocarps and cultures. Only four of these 10 matching pairs were identified to species level by DNA sequence analysis.
- Only 30 sequences out of 57 obtained directly from sporocarp tissue supported at some level (seven to species level) the original morphological identifications attributed to the sporocarps analysed. Eleven sporocarp sequences and 12 isolate sequences, obtained from 19 sporocarps identified to species or genus level did not support the morphological identifications.

There was however the benefit of molecular identifications for sporocarps in the absence of morphological identifications. Sequences were derived from 15 of 27 unidentified sporocarps and there were seven identifications to species or genus level which could possibly have been correct in respect to the type of sporocarp collected. It is a bit puzzling that, since the objective was to investigate wood samples

The study showed that where a large number of wood decay isolates are obtained during an investigation that molecular analyses are useful for the following:

- confirming, refining or reallocating the identity of isolates that have characteristic morphology in rotting wood or in culture - either by direct comparison to known sequences and/or phylogenetic analysis e.g. for 11 isolates that had been identified as *Polyporus mylittae* (since renamed *Laccocephalum mylittae)*. The closest BLAST match results for those isolates were *Diplomitoporus lindbladii* (currently accepted as *Poria lindbladii*) and *Postia subcaesia* (currently accepted as *Tyromyces subcaesius*), though no sequences of *L. mylittae* or other *Laccocephalum* species were available for comparison. A herbarium specimen of *L. mylittae* was obtained and the ITS sequenced, but this sequence did not match that of any of the 11 isolates. These 11 isolates were therefore accepted as belonging to a different species in the Polyporaceae.
- identifying isolates obtained from rotting wood and which have been morphologically grouped according to their cultural characteristics e.g 4 isolates grouped as *Basidio*-sp.6 were identified to genus level, *Antrodia.*

Twenty-six samples of rotten wood taken from 19 decayed logs at six sites gave rise to a total of 80 DNA samples but only 17 PCR products and 10 readable sequences. A cloning step to separate the different fungal templates before sequencing was necessary in the majority of samples (Trang *et al.* 2006) and this adds to the cost of the process. Three wood samples originating from one type of rotting wood yielded very different fungi including the groups of Basidiomycota, Ascomycota and even Zygomycota. As a sample of rotting wood will contain multiple fungi this result is expected.

Eight different fungi were identified but with very low levels of confidence, from the rotten wood samples, six were Basidiomycetes. However, the fungi identified did not match the original fungi cultured from these samples, probably because a) many of the 93 original cultures had become contaminated in storage and b) the wood samples had degraded in cold storage. The only basidiomycete identified with any level of confidence was a *Ganoderma* species that in phylogenetic analysis was close to a species from oil palm, indicating possible contamination of DNA samples.

Despite limitations, molecular analyses of this type will eventually allow the construction of a sequence reference library and the importance of this study is to highlight the problems and pitfalls involved as follows:

Strategies for the improvement of molecular tools to identify wood decay fungi

- There are several compounding reasons which can be addressed for the low rate of success in obtaining identified sequences for a reference collection.

  - The success rate of isolations obtained from sporocarps is approximately 70%. Isolates cultured may be a contaminant of the sporocarp – approximately 41% of DNA sequences for the 56 fungal isolates supported the morphological identification of the sporocarps from which the fungal isolates had been cultured (*use improved selective media for basidiomycetes, isolate from a young sporocarp and internal tissue that is less likely to be contaminated, use enzyme tests to confirm wood rotting capacity, examine the culture under the microscope for characteristic features that either confirm or eliminate the possibility of a wood decay fungus e.g. clamps or conidiophores*).
  - There was a high level of contamination of the 93 cultures (identified at some level) from a previous study of wood decay fungi and which were to have provided a source of reference sequences. This contamination had probably taken place during storage and routine subculturing, as the molecular identification bears no relation to the original morphological grouping e.g. isolates identified as Basidiomycetes were identified by sequencing as Xylariaceae. The contamination probably took place during storage and routine subculturing; as the molecular identification often bore no relation to the original morphological grouping e.g. isolates identified as Basidiomycetes were identified by sequencing as Xylariaceae. This is always going to be a problem with large-scale studies resulting in over 1000 cultures. (*This may be overcome in the future by grouping isolates through enzyme tests and a quick morphological examination, then keeping only one or two representative*

isolates but DNA of other isolates in the same group or DNA analysis before the cultures are destroyed).

- There is little expertise available to morphologically identify sporocarps (only 84 out of 111 sporocarps were morphologically identified to species or genus level before DNA analysis). Even experts find the morphological identification of wood decay fungal sporocarps difficult. (*Where possible get confirmation from a mycologist or until this can happen, which may take several years, ensure that a proper herbarium specimen is retained and given an adequate description and tag name*).
- DNA is not successfully extracted from the sporocarps and/or cultures, either not at all or with high concentrations of compounds that inhibit molecular analyses (*improve extraction techniques by using commercial purification kits*).
- The primers that are used may not amplify all fungal species (*try different primer sets for the same region*).
- Contaminant DNA is obtained from sporocarps meaning that there are multiple PCR products which interfere with the readability of sequencing results (*in this case cloning would be required before sequencing*).
- In addition to all the above limitations there may be no matching sequences available in public databases as Australian wood decay fungi have not been widely studied. Sequences may not be reliable if present as many sequences submitted to Genbank do not include isolate of herbarium accession details. For example, the sporocarps E7601, E7554 and isolates LF5, LF67 do not have corresponding sequences of *Ryvardenia*, *Fomes hemitephrus*, *Xylobulus illudens* and *Postia caesia* respectively available on both Genbank and private databases for comparison. (*Care should be taken when using sequences from public databases, the poor availability will probably take several years to improve and can be started by the Hobart Forest health group making their sequences available*).

➢ The major issue with the identification of fungi directly from rotten wood was the non-amplification of DNA. The by-products of wood decay most likely prohibited amplification of the DNA template (Jasalavich *et al.* 2000). Diluting out the inhibitors could also mean diluting out the DNA past the threshold of detection (Jasalavich *et al.* 2000). Amplication of two samples was successful after purification of the fungal DNA using Polyvinylpolypyrrolidone (PVPP). The low rate of success in identifying fungi directly from rotten wood samples is also attributable to the fact that these samples were not processed immediately they were harvested or frozen at -80 °C and then processed. In this study, the samples were left at 4 °C before analysis and it is likely that, even at this low temperature, the basidiomycete fungi present were out competed by other fungi. Further analyses to detect and identify fungi direct from wood will be carried out. This pilot study to directly extract fungi from rotted wood material was a useful learning experience.

5. Conclusions

This pilot study to develop molecular tools to detect and identify wood decay fungi directly rotten wood material in decayed logs clearly illustrated some of the difficulties in taking this route, especially that imposed by a lack of classical taxonomic expertise.

There are a number of interlocking contradictions regarding the specificity of the diagnostic sequences and the ability to classify fruiting bodies and other morphological traits with certainty i.e.

- ✔ Fungal taxonomy is poorly developed
- ✔ Only 5% of fungi in Australia may be classified and described in detail
- ✔ The level of intraspecific heterogeneity varies between species
- ✔ Intra-generic homogeneity has been observed
- ✔ Australian fungi are under represented in sequence databases

The strategy adopted in this thesis to identify wood decay fungi could be compromised by any of the above.

The study of wood decay fungi in this thesis however proved a valuable exercise to highlight problems associated with the development of molecular identification and detection tools for wood decay fungi so that these problems can be mitigated in future research. As a sample of rotting wood is a microcosm of fungi, containing not just one or several basidiomycete wood-decay fungi, an investigation of specific species (e.g. *Ganoderma* spp.) will require the development of fungal specific primers. If only an idea of the different groups of fungi present is required then an analytical technique such as T-RFLP should be employed with relevant fungal specific primers (e.g. Allmér *et al.* 2006). The molecular approach adopted will depend upon the precise ecological question being explored. Whatever the approach this study highlighted that there must be a good integration between molecular and conventional mycological methodologies involved in studying wood decay fungi – no one technique by itself can give coherent or useable results.

In the future, it is planned to use detection direct from wood to undertake large multi-scale studies to examine the association of specific fungal species and saproxylic beetles with particular rot types, the successional pathways followed by these organisms in native and logging regenerated forest. This type of research will yield information to assist in the development of management prescriptions to prevent loss of coarse wood debris (CWD) dependent biodiversity in the forest production landscape.

Section 3: General Discussion and Conclusions

Fungal diseases have had a marked impact on eucalypt productivity in Vietnam and the area of plantation under *Eucalyptus*. Breeding programs have been implemented to address the problem of disease and have focused on the selection of species or clonal lines which have both high yield and disease resistance. Resistance screening was carried out in three clonal eucalypt trials planted in southern Vietnam assessing sixty eucalypt clones and one seedlot (a landrace as a control) for growth traits, survival and crown damage. Approximately half of the clones, including the control, were classed as poorly performing clones based on the various criteria. Seventeen clones were significantly susceptible to disease at one or more sites. The clones ranked as top performers were those that were fast growing, with a high survival rate. *Coniella* leaf spot was prevalent but was not systematically associated with high levels of damage. The more damaging pathogens present were *Cryptosporiopsis eucalypti*, *Cylindrocladium quinqueseptatum*, *Kirramyces destructans*. *Microsphaeropsis globulosa* on eucalypts was recorded for the first time on *Eucalyptus* in Vietnam. The top performing clones were apparently resistant to disease although this conclusion must be treated with caution. Natural infection resulted in a different suite of fungal leaf pathogens at each site. Disease incidence and severity was also different at each site. Disease resistance could therefore be associated with vigour rather than actual genetic resistance. This observed disease resistance or tolerance might not be expressed against a new disease incursion or an increased pathogenicity in the populations of fungal leaf pathogens.

Although the major fungal leaf pathogens were usually easily recognisable in this study there were ambiguities in identification in the field explained by the presence of un- or poorly described species. Also it is often difficult to accurately identify a pathogen especially in the early stages of its infection before there is a complete expression of diagnostic symptoms.

It may be a tedious, time consuming and costly process to isolate pathogens from tissue. Conventional methods for the positive identification of these and other leaf pathogens involve waiting until lesions produce ‘typical’ reproductive structures or forms, isolating them, and identification of putative pathogens using taxonomic criteria. Identification is further confounded by the fact that more than species may be present in tissue or a single lesion. Other limitations of conventional methods may include low isolation frequencies from diseased material. Future resistance screening and associated epidemiological studies will benefit from molecular techniques to detect and identify fungi, especially methods that can be applied directly to plant tissue.

Before attempting to identify fungi directly from the fragments of decayed log, a reference library of ITS sequences was established from the DNA analysis of 93 wood decay isolates obtained from a previous study (which had been grouped according to morphology) and 111 sporocarps. Sporocarps were morphologically identified to a genus or species level or had been given a tag name while awaiting formal identification. rDNA was amplified from DNA extracted from sporocarp tissue and fungal cultures. The internal transcribed spacer (ITS) region of the rDNA was sequenced.

Out of 111 sporocarps and 70 isolates obtained from these sporocarps, sequencing definitively coupled only 10 pairs of sporocarps and cultures. Only four of these 10 matching pairs were identified to species level by DNA sequence analysis. Only 30 sequences out of 57 obtained directly from sporocarp tissue supported at some level (seven to species level) the original morphological identifications attributed to the sporocarps analysed. Eleven sporocarp sequences and 12 isolate sequences, obtained from a total of 19 sporocarps which gave species or genus level identifications did not support the morphological identifications. There was a high level of contamination of the 93 cultures from a previous study of wood decay fungi. This contamination had probably taken place during storage and routine subculturing, as the molecular identification bears no relation to the original morphological grouping e.g. isolates identified as Basidiomycetes were identified by sequencing as Xylariaceae. Despite obvious limitations to this pilot study, molecular analyses of this type will eventually allow the construction of a sequence reference library for wood decay fungi. The importance of this study was to highlight the problems and pitfalls involved in establishing a sequence reference library which will be required for any study where a large number of fungal species will be present in the environment to be investigated.

DNA was extracted from fragments of rotted wood from *E. obliqua* logs and the rDNA ITS amplified with fungal specific primers. The PCR products were cloned before sequencing as multiple fungal species were present in most samples. Clones were screened by PCR-RFLP and representatives of each PCR-RFLP group were sequenced. To identify the fungi present in the rotted wood samples, sequences were compared to those from public and private databases, including the reference database set up within this study.

The major issue with the identification of fungi directly from rotten wood was the non-amplification of DNA. Twenty-six samples of rotten wood taken from 19 decayed logs at six sites gave rise to a total of 80 DNA samples but only 17 PCR products and 10 readable sequences. Three wood samples originating from one type of rotting wood yielded very different fungi including the groups of Basidiomycota, Ascomycota and even Zygomycota. As a sample of rotting wood is a microcosm of fungi, not just one or several basidiomycete wood-decay fungi, and the primers used were fungal specific not basidiomycete specific, this result is expected. Eight different fungi were identified but with very low levels of confidence, from the rotten wood samples, six were Basidiomycetes. However, the fungi identified did not match the original fungi cultured from these samples. Despite ambiguous results this project has proved valuable as a pilot study for understanding the problems involved in the detection of fungi directly from various substrates such as wood or leaves.

Because of quarantine restrictions, it was difficult to bring diseased leaf samples from the Vietnamese resistance trials assessed in Section 1 into Hobart. This was attempted but the method used to comply to quarantine restrictions degraded DNA and made its extraction difficult; the infected plant tissue (plant tissue was soaked in 70% alcohol for 2 weeks and then transferred to sterile water) and subsequent DNA extractions from the tissue were not successful. To develop suitable detection methodology this study used material easily available in Hobart. It was considered that the direct detection and identification of fungi direct from material taken from rotting logs found in the *Eucalyptus obliqua* wet sclerophyll forests of southern Tasmania would illustrate both conceptually and empirically the difficulties of direct detection and identification of fungi from eucalypt leaves in the tropics. In both the leaf and log materials many unknown biotic organisms especially fungi will be present (these may not necessarily be pathogens, possibly endophytes or saprophytes).

The development of molecular tools for application to forestry in Vietnam will support fundamental research (e.g. enabling studies to more fully understand a pathogen, its etiology, biology and epidemiology). This information is critical to the development of resistance as a disease management strategy, especially if clones are screened under conditions of natural infection, where it is difficult to compare the performance of clones unless the fungal pathogens causing damage are determined accurately. Additionally, the development of resistance as a disease management strategy will often require for germplasm movement within a country or between countries. Plant quarantine risks involved with global germplasm movement can be reduced with the assistance of molecular based diagnostic tools. These have wide application to ensuring that the trade of germplasm between countries is biosecure and to the certification of germplasm internationally, nationally and regionally. The tools will often be used to confirm diagnosis when an incursion is suspected. If existing tools are further developed with microarray technology they can be applied to the broad scale quarantine surveillance of pathogens at high risk sites such as port environs. If a disease was detected and confirmed at an early stage, disease eradication would be possible.

In conclusion, this thesis investigated the methodologies, advantages and problems associated with identifying fungi directly from plant tissue using molecular techniques. Assessments of 'disease resistance' in eucalypt clones under conditions of natural infection in the field reliably indicated the performance of clones but mainly in terms of vigour. The quality of information about disease resistance in the screening trials could have been improved by the use of molecular tools, similar to those applied to the study of wood decay fungi in this thesis, to accurately identify the pathogens present on top or poorly performing clones.

References

Akiew, E. and Trevorro, P. R. (1994) Management of bacterial wilt of tobacco. In: Bacterial Wilt: The Disease and Its Causative Agent, *Pseudomonas solanacearum.* (eds Hayward, A. C. and Hartman, G. L.) 179-198 pp. CABI, Wallingford, UK.

Allmér, J., Vasiliauskas, R., Ihrmark, K., Stenlid, J. and Dahlberg, A. (2006) Wood-inhabiting fungal communities in woody debris of Norway spruce (*Picea abies* (L.) Karst.), as reflected by sporocarps, mycelial isolations and T-RFLP identification. FEMS *Microbiology Ecologist* 55: 57-76.

Altschul, S. F., Madden, T., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389-3402.

Andjic, V., Barber, P. A., Carnegie, A. J., Hardy, G. E. S. J., Wingfield, M. J., Burgess, T. I. (2007) A morphological and phylogenetic reassessment of the genus *Phaeophleospora* and the resurrection of the genus *Kirramyces*. *Mycological Research* (accepted)

Andrianova, T. V. and Minter, D. W. (2005) *Coniella australiensis*. In: Descriptions of Fungi and Bacteria, No. 163, sheet 1622.

ANGIS (2005) BioManager by ANGIS: Australian National Genome Information Services. 2005, http://www.angis.org.au.

Banh, T. H. (1999) Eucalypt plantations and their disease situation in Thu Thien Hue province. ACIAR Workshop on Eucalypt Diseases, Ho Chi Minh City, Vietnam, 8-10 November 1999 (Abstract in Workshop booklet).

Blum, L. E. B., Dianese, J. C. and Costa, C. L. (1992) Comparative pathology of *Cylindrocladium clavatum* and *C. scoparium* on *Eucalyptus* spp. and screening of *Eucalyptus* provenances for resistance to *Cylindrocladium* damping-off. *Tropical Pest Management* 38: 2155-2159.

Bolland, L. Tierney, J. W. and Tierney, B. J., (1985) Studies on leaf spot and shoot blight of *Eucalyptus* caused by *Cylindrocladium quinqueseptatum*. *European Journal of Forest Pathology* 15: 385-397.

Booth, T. H. (1996) Matching Trees and Sites. Proceedings of an International Workshop held in Bangkok, 27-30 March 1995 (ACIAR Proceedings No.63). ACIAR, Canberra, Australia.

Booth, T. H., Jovanovic, T., Old, K. M., and Dudzinski, M. J. (2000) Climatic mapping to identify high-risk areas for *Cylindrocladium quinqueseptatum* leaf blight on eucalypts in mainland South East Asia and around the world. *Environmental Pollution* 108: 365-372.

Booth, T. H., Stein, J. A., Nix, H. A. and Hotchinson, M. F. (1989) Mapping regions climatically suitable for particular species: an example using Africa. *Forest Ecology and Management* 28: 19-31.

Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1996) Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research Monograph 32, Canberra, Australia.

Burgess, T. I., Andjic, V., Hardy, G. E. S., Dell, B. and Xu, D. (2006) First report of *Phaeophleospora destructans* in China. *Journal of Tropical Forest Science* 18: 144-146.

Ciesla, W. M., Diekmann, M. and Putter, C. A. J. (1996) *Eucalyptus* spp. FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm. No.17. Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome, Italy.

Coutinho, T. A., Wingfield, M. J., Roux, J., Riedel, K. H. and Terblanche, J. (2000) First report of bacterial wilt caused by *Ralstonia solanacearum* on *Eucalyptus* in South Africa. *European Journal of Forest Pathology* 30: 205-210.

Cromer, R. N., Ryan, P. A., Booth, T. H., Cameron, D. M. and Rance, S. J. (1991) Limitations to productivity of *Eucalyptus grandis* plantations in sub-tropical Australia.

In: Productivity in Perspective (ed Ryan, P. J) 133-146 pp. Proceedings of the Third Australian Forest Soils and Nutrition Conference, Melbourne, Australia.

Crous, P. W. (1998) *Mycosphaerella* spp. and their anamorphs – associated with leaf spot disease of *Eucalyptus*. *Mycologia Memoir* 21: 170.

Crous, P. W. (2002) Taxonomy and Pathology of *Cylindrocladium (Calonectria)* and Allied Genera. APS Press, St Paul, Minnesota, USA.

Crous, P. W., Knox-Davies, P. S. and Wingfield, M. J. (1989) A list of *Eucalyptus* leaf fungi and their potential importance to South African forestry. *South African Forestry Journal* 149: 17-29.

Crous, P. W., Phillips, A. J. L. and Wingfield, M. J. (1993) New records of *Cylindrocladium* and *Cylindrocladiella* spp. in South Africa. *Plant Pathology* 42: 302-305.

Crous, P. W. and Wingfield, M. J. (1994) A monograph of *Cylindrocladium,* including anamorphs of *Calonectria*. *Mycotaxon* 51: 341-453.

Crous, P. W., Wingfield, M. J., Mansilla, J. P., Alfenas, A. C. and Groenewald, J. Z. (2006) Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. II. *Studies in Mycology* 55: 99-131.

Cunningham, G. H. (1965) Polyporaceae of New Zealand. New Zealand Department of Scientific and Industrial Research, Bulletin 164, Wellington.

Dianese, J. C., Dristig, M. C. G. and Cruz, A. P. (1990) Susceptibility to wilt associated with *Pseudomonas solanacearum* among six species of *Eucalyptus* growing in equatorial Brazil. *Australasian Plant Pathology* 19: 71-76.

Dick, M. A., Dobbie, K., Cooke, D. E. and Brasier, C. M. (2006) *Phytophthora captiosa* sp. nov. and *P. fallax* sp. nov. causing crown dieback of *Eucalyptus* in New Zealand. *Mycological Research* 110: 393-404.

Dudzinski, M. J., Old, K. M., Thu, P. Q., Nguyen, T. N., and Gibbs, R. J. (1999) Selecting *Eucalyptus camaldulensis* resistant to foliar diseases at Chon Thanh, Vietnam. ACIAR 9441 Workshop on Eucalypt Diseases, Ho Chi Minh City, Vietnam, 8-10 November 1999 (Abstract in Workshop booklet).

FAO (2007) Overview of Forest Pests in Morocco. FAO, Rome, Italy.

Farmer, D. J. and Sylvia, D. M. (1998) Variation in the ribosomal DNA internal transcribed spacer of a diverse collection of ectomycorrhizal fungi. *Mycological Research* 102: 859-865.

Felsenstein, J. (1989) PHYLIP – Phylogeny Inference Package (Version 3.2). *Cladistics* 5: 164-166.

Forest Inventory and Planning Institute (FIPI) (2005) Distribution of Forest Plantation Areas and Forest Reserve in Vietnam 2005. Agricultural Publishing House, Hanoi, Vietnam.

Gadgil, P. D. and Dick, M. (1999) Fungi silvicolae novazelandiae: 2. *New Zealand Journal of Forest Science* 29: 440-458.

Gadgil, P. D., Wardlaw, T. J., Ferreira, F. A., Sharma, J. K., Dick, M. A. Wingfield, M. J. and Crous, P. W. (2000) Management of disease in eucalypt plantations. In: Diseases and Pathogens of Eucalypts. (eds Keane, P. J., Kile, G. A., Podger, F. D. and Brown, B. N.) 519-529 pp. CSIRO Publishing, Collingwood, Vic, Australia.

Gardes, M. and Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes – Applification to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118.

GenStat (2003) GenStat for Windows. Release 7.1. Seventh Edition. VSN International Ltd., Oxford.

Glen, M., Tommerup, I. C., Bougher, N. L. and O'Brien, P. A. (2002) Are Sebacinaceae common and widespread ectomycorrhizal associates of *Eucalyptus* species in Australian forests? *Mycorrhiza* 12: 243-247.

Glen, M., Tommerup, I. C., Bougher, N. L. and O'Brien, P. A. (2001) Specificity, sensitivity and discrimination of primers for PCR-RFLP of larger basidiomycetes and their applicability to identification of ectomycorrhizal fungi in *Eucalyptus* forests and plantation. *Mycological Research* 105: 138-149.

Glen, M., Alfenas, A. C., Zauza E. A. V., Wingfield, M. J. and Mohammed C. (2007a) *Puccinia psidii*: a threat to the Australian environment and economy – a review. *Australian Plant Pathology* 36: 1-16.

Glen, M., Smith, A. H., Langrell, S. R. H. and Mohammed, C. L. (2007b) Development of nested polymerase chain reaction detection of *Mycosphaerella* spp. and its application to the study of leaf disease in *Eucalyptus* plantations. *Phytopathology* 97: 132-144.

Grove, S. J., Meggs, J. and Goodwin, A. (2002) A Review of Biodiversity Conservation Issues Relating to Coarse Wood Debris: Management in the Wet Eucalypt Production Forests of Tasmania. Report for Forestry Tasmania, Hobart, Australia.

Hawksworth, D. L. (1991) The fungal dimension of biodiversity: magnitude, significance and conservation. *Mycological Research* 95: 641-655.

Heilmann-Clausen, J. and Christensen, M. (2003) Fungal diversity on decaying beech logs – implications for sustainable forestry. *Biodiversity and Conservation* 12: 953-973.

Hood, I. A. (2003) An Introduction to Fungi on Wood in Queensland. Published by University of New England, Armidale, Australia.

Hopkins, A. J. M. (2006) The taxonomy and ecology of wood decay fungi in *Eucalyptus obliqua* trees and logs in the wet sclerophyll forests of southern Tasmania. PhD Thesis, University of Tasmania, Australia.

Horton, T. R. and Bruns, T. D. (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10: 1855-1871.

Jasalavich, C. A., Ostrofsky, A. and Jellison, J. (2000) Detection and identification of decay fungi in spruce wood by Restriction Fragment Length Polymorphism analysis of amplified genes encoding rRNA. *Applied and Environmental Microbiology* 66: 4725-4734.

Jayasree, M. C., Nair, J. M., Deo, A. D. Ramaswamy, V., Sharma, J. K., Nair, C. T. S., Kedharnath, S. and Kondas, S. (1986) Relative susceptibility of various eucalypt provenances to Cylindrocladium blight. In: Proceedings of the Seminar on *Eucalyptus* in India: Past, Present and Future. (eds Sharma, J. K., Nair, C. T. S., Kedharnath, S., Kondas, S.) 395-399 pp. Held at Kerala Forest Research Institute, 30-31 January 1984 Peechi, Kerala, India.

Hoff, J. A., Klopfenstein, N. B., Tonn, J. R., McDonald, G. I., Zambino, P. J., Rogers, J. D., Peever, T. L., and Carris L. M. (2004) Roles of woody root-associated fungi in forest ecosystem processes: Recent advances in fungal identification. Research Paper RMRS-RP-47. Fort Collins, Co: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, 6 pp.

Johannesson, H. and Stenlid, J. (1999) Molecular identification of wood inhabiting fungi in an unmanaged *Picea abies* forest in Sweden. *Forest Ecology and Management* 115: 203-211.

Kårén, O., Högberg, N., Dahlberg, A., Jonsson, L. and Nylund, J. E (1997) Inter-and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. *New Phytologist* 136: 313-325.

Kha, L. D. (1996) Research on Formulation of Scientific and Technological Basis for Supplying Improved Planting Material of Forest Trees. Scientific report of the research program KN 03-03. Forest Science Institute of Vietnam 53 pp.

Kha, L. D. (1998) Tree selection for economic plantations in south-east Vietnam. Paper to the Conference on Forest Science Institute of Vietnam Research Results in South-East Vietnam, 1998. Forest Sicence Institute of Vietnam, 8 pp.

Kile, G. A. and Johnson, G. C. (2000) Stem and butt rots of eucalypts. In: Diseases and Pathogens of Eucalypts. (eds Keane, P. J., Kile, G. A., Podger, F. D. and Brown, B. N.) 307-338 pp. CSIRO publishing, Collingwood, Australia.

Kirkpatrick, J. B. (1988) The West Eucalypt Forests of Tasmania. Tasmanian Conservation Trust, Hobart, Australia.

Kreader, C. A. (1996) Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and Environmental Microbiology* 62: 1102-1106.

Machmud, M. (1985) Bacterial wilt in Indonesia. In: Bacterial Wilt Disease in Asia and the South Pacfic. (eds Craswell, E. T and Pushperajah, E.) 30-34 pp. ACIAR Proceedings 13. ACIAR, Canberra, Australia.

Mao, T.V. (1994) Pathological Evaluation of Nurseries and Plantations in the 13 coastal provinces involved in World Food Program (WFP) Project 4304, United Nations Development Program /FAO (Vietnam: Provision of Technical Support to WFP /92/022).

MARD (Ministry of Agriculture and Rural Development) (2002) Statistics of Agriculture and Rural Development 1996-2000. Agricultural Publishing House, Ha Noi, Vietnam 599 pp.

May, T. W. (2001) Documenting the fungal biodiversity of Australia from 1980 to 2000 and beyond. *Australian Systematic Botany* 14: 329-356.

May, T. W. and Pascoe, I. G. (1996) History of the taxonomic study of Australian fungi. In: Fungi of Australia 1A, Introduction – Classification. (ed. Orchard A. E.) 171-206 pp. Australian Biological Resources Study, Canberra, Australia.

Mendel, Z., Protasov, A., Fisher, N. and Salle, L. S. (2004) Taxonomy and biology of *Leptocybe invasa* gen. & sp. nov. (Hymenoptera: Eulophidae), an invasive gall inducer on *Eucalyptus*. *Australian Journal of Entomology* 43: 101-113.

Mitchell, J. I., Roberts, P. J., and Moss, S. T. (1995) Sequence or structure? A short review on the application of nucleic acid sequence information to fungal taxonomy. *The Mycologist* 9: 67-75.

Mohammed, C. L. and Yuan, Z. Q. (2002) Report for Project No. C199067: Ecologically Sustainable Forest Management: Fungal and Invertebrate Biodiversity. ARC SPIRT Project 1999-2002, Hobart, Australia.

Mutitu, K. E. (2003) A Pest Threat to *Eucalyptus* Species in Kenya. Kenya Forest Research Institute Technical Report. 12 pp.

Bureau of Rural Science (2005) National Forest Inventory Australia's State of the Forests Report, Canberra, Australia.

Nghia N. H (1992) Structure of *Eucalyptus* species and actual fungal disease problems. *Forestry Review* 5: 24-26.

Nghia, N. H. (1999) Eucalypt Selection for Growth and Disease Resistance. Internal Report No. 24, Forest Science Institute of Vietnam.

Nghia, N. H. (2000) Selection of *Eucalyptus* species according to growth and disease resistance in Vietnam. Agricultural Publishing House, Hanoi, Vietnam 112 pp.

Nghia, N. H. (2003) Selection of Disease Tolerant Varieties with High Productivity for Eucalypts and Acacias in 2001-2002 period. Hanoi, Forest Science Institute of Vietnam, scientific report, 30 pp. (in Vietnamese).

Nghia, N.H. (2004) Report on Eucalypt in Vietnam. Internal Report No. 15, Forest Science Institute of Vietnam.

Nghia, N. H. (2006) Report on Results of the Project "Screening for the Selection of *Eucalyptus* and *Acacia* Species for High Productivity and Disease Resistance" period 2001 and 2005. Internal Report, Forest Science Institute of Vietnam.

Nghia, N. H. and Old, K. M. (1997) Variation in growth and disease resistance of *Eucalyptus* species and provenances tested in Vietnam. In: Silviculture and Genetic Improvement of Eucalypts. (eds Hig, A. R., Shcaitza, E. and Gaiad, S.) 416-422 pp. Proceedings of IUFRO conference, Brazil, 1997.

Nguyen, T. N. (1999) Early Rrowth and Disease Assessment of a *Eucalyptus camaldulensis* Progeny Trial in the South-East Vietnam. Australian Tree Seed Centre, CSIRO Forestry and Forest Products, Canberra, Australia. Professional Attachment Report 53 pp.

Nobles, M. K. (1948) Studies in forest pathology VI. Identification of cultures of wood-rooting fungi. *Canadian Journal of Forest Research* 26: 281-431.

Nyeko, P. (2004) The occurrence and severity of a new and threatening gall damage on *Eucalyptus* species in Uganda. In: Pest Management in Tropical Plantations. (eds Cobbinah, J. R., Ofori, D. A., and Bosu, P. P.) 100-107 pp. Kumasi, Ghana: University Printing Press (UPK), KNUST.

Nyeko, P., Mutitu, E. K. and Day R. K. (2007) Farmers' knowledge, perceptions and management of the gall-forming wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae), on *Eucalyptus* species in Uganda. *International Journal of Pest Management* 53: 111-119.

Old, K. M. (1999) Minimising Disease Impacts on Eucalypts in South East Asia. Fourth annual progress report CSIRO/ACIAR project 9441. CSIRO Forestry and Forest Products, Canberra, Australia.

Old, K. M. (2001) Minimising Disease Impacts on Eucalypts in South East Asia. Final report CSIRO/ACIAR project 9441. CSIRO Forestry and Forest Products, Canberra.

Old, K. M., Nghia, N. H., Dudzinski, M. J., Thu, P. Q., Nguyen, N. T., Chien, N. V. and Gibbs, R. J. (2000a) Selection of clones of *Eucalyptus camaldulensis* resistant to leaf blight in Vietnam. ACIAR Workshop on Eucalypt Diseases and Their Management, Bangkok, Thailand, 6-8 November 2000 (Abstract in Workshop booklet).

Old, K. M., Thu, P. Q., Dudzinski, M. J. and Gibbs, R. J. (2000b) *Eucalyptus* pathology in Vietnam. ACIAR Workshop on Eucalypt Diseases and Their Management, Bangkok, Thailand, 6-8 November 2000 (Abstract in Workshop booklet).

Old, K. M., Dudzinski, M. J., Pongpanich, K., Yuan, Z. Q., Thu, P. Q., and Nguyen, T. N. (2002) Cryptosporiopsis leaf spot and shoot blight of eucalypts. *Australasian Plant Pathology* 31:337-344.

Old, K. M., Dudzinski, M. J. and Thu, P. Q. (1999) Laboratory Workbook for the Technical Workshop on Eucalypt Diseases. ACIAR 9441 Minimising Disease Impacts on Eucalypts in South-East Asia, Technical Workshop, Ho Chi Minh City, 11-12 November 1999. 21 pp. (English and Vietnamese Versions).

Old, K. M., Pongpanich K., Thu, P. Q. Wingfield, M. J. and Yuan Z. Q. (2003a) *Phaeophleospora destructans* causing leaf blight epidemics in South-East Asia. In: Proceedings of 8th International Congress of Plant Pathology, 2-7 February 2003, Christchurch, New Zealand, V2, 65 pp.

Old, K. M., Wingfield, M. J. and Yuan, Z. Q. (2003b) A Manual of Diseases of Eucalypts in South-East Asia. Centre for International Forestry Research. Jakarta 10065, Indonesia.

Old, K.M. and Yuan, Z.Q. (1995) Foliar and Stem Disease of Eucalyptus in Vietnam and Thailand. Internal Report, CSIRO Division of Forestry and Australian Centre for Agricultural Research, Canberra, Australia. 15pp.

Page, R. D. M. (2001) TreeView (Win 32) Version 1.6.6 http://taxonomy.zoology.gla.ac.uk/rod/rod.html accessed date: 10 May 2007.

Pearson, W. R. and Lipman, D. J. (1998) Improved Tools for Biological Sequence Analysis. Proceedings of the National Academy of Science, USA 85: 2444-2448.

Peerally, A. (1974) *Calonectria quinqueseptata* (conidial state: *Cylindrocladium quinqueseptatum*). CMI Descriptions of Pathogenic Fungi and Bacteria No. 423, 2 pp.

Pegg, G., Brown, B. and Ivory, M. (2003) Eucalypt Disease in Hardwood Plantations in Queensland. Hardwoods Queensland Report no. 16. Forestry Research, Agency for Food and Fibre Sciences, DPI.

Pitkethley, R. N. (1976) *Cylindrocladium quinqueseptatum* on myrtaceous tree seedlings. *Australian Plant Pathology Society Newsletter* 5: 57.

Pongpanich, K. (2000) *Eucalyptus* Pathology in Thailand. In: Eucalypt Diseases and Their Management. Proceedings of a workshop, Kasetsart University, Bangkok 6-8 November 2000. ACIAR 9441 Final Report. Attachment 4.

Purnell, R. C. and Lundquist, J. E. (1986) Provenance variation of *Eucalyptus nitens* on the Eastern Transvaal Highveld in South Africa. *South African Forestry Journal* 138: 23-31.

Raeder, U. and Broda, P. (1985) Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* 1: 17-20.

Sankaran, K.V., Sutton, B. C. and Balasundaran, M. (1995) *Cryptosporiopsis eucalypti* sp. nov. causing leaf spots of eucalypts in Australia, India and USA. *Mycological Research* 99: 827-830.

Sharma, J. K. (1994) Pathological Investigations in Forest Nurseries and Plantations in Vietnam. FAO consultancy report, UNDP/FAO Project VIE/92/022. Hanoi, Vietnam. 46 pp. (English and Vietnamese versions).

Sharma, J. K. and Mohanan, C. (1982) *Cylindrocladium* spp. associated with various diseases of *Eucalyptus* in Kerala. *European Journal of Forest Pathology* 12: 129-136.

Sharma, J. K. and Mohanan, C. (1992) Relative susceptibility of *Eucalyptus* provenances to Cylindrocladium leaf blight in Kerala, India. *European Journal of Forest Pathology* 22: 257-265.

Sharma, J. K. Mohanan, C., Florence, E. J. M. (1985) Disease Survey in Nurseries and Plantation of Forestry Tree Species Grown in Kerala (Reseach report No. 36). Kerala Forest Research Institute, Peechi, India.

Sharma, J. K., Balasundaran, M. and Florence, E. J. M. (1999) Increasing the productivity of eucalypts in Kerala through selection for diseases resistance, higher growth and clonal technology. In: Plant Protection in the Tropics: Tropical Plant Protection in the Information Age. (eds Sivapragasam, A., and Loke W. H.) 164-167 pp. Proceedings of the Fifth International conference of the Malaysian Plant Protection society, 15-18 March 1999, MPPS, Kuala Lumpur, Malaysia.

Simpson, J. A. (1996) Wood decay fungi. In: Fungi of Australia 1B, Introduction-Fungi in the Environment. (ed. Orchard, A. E.) 95-136 pp. Australian Biological Resources Study, Canberra, Australia.

Smith, A. (2006) The development of strategies for the management and research for foliar pathogens on *Eucalyptus* plantation: Using *Mycosphaerella* as a case study. PhD Thesis, University of Tasmania, Australia.

Smith, I. M., McNamara, D. G., Scott, P. R. and Harris, K. M. (eds) (1992) Quarantine Pests for Europe: Data Sheets on Quarantine Pests for the European Communities and for The European and Mediterranean Plan Protection Organization. CAB International, Wallingford, UK, and Eurpean and Mediterranean Plant Protection Organization, Paris 1425 pp.

Sutton, B. C. (1980) The *Coelomycetes*. Commonwealth Mycological Institute, Kew, 696 pp.

Thiep, T. X. (1996) *Eucalyptus* Plantation in Vietnam: Their History and Development Process. In: Reports Submitted to the Regional Expert Consultation on *Eucalyptus*. Volume II. (eds Kashio, M. and White, K.). FAO Regional Office for Asia and the Pacific (RAP) Bangkok, Thailand 1996.

Thompson, J. D., Higgins, D. G and Gibson, T. J. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680.

Thu, P. Q., Old, K. M., Dudzinski, M. J. and Gibbs, R. J. (2000) Result of eucalypt disease surveys in Vietnam. ACIAR Workshop on Eucalypt Diseases and Their Management, Bangkok, Thailand, 6-8 November 2000 (Abstract in Workshop booklet).

Thu, P. Q. (2002) Diseases of eucalypts and epidemic control measures. *Science and Technology Journal of Agriculture and Rural Development* 4: 330-331.

Thu, P. Q. (2004) A strange insect damaging *Eucalyptus* has occurred on our country. *Science and Technology Journal of Agriculture and Rural Development* 11: 1598-1599.

Thu, P. Q. (2005a) *Eucalyptus* leaf spot, top-drying disease caused by *Cryptosporiopsis eucalypti*. *Science and Technology Journal of Agriculture and Rural Development* 8: 67-68.

Thu, P. Q. (2005b) *Eucalyptus* leaf spot in nurseries and plantations caused by *Phaeophleospora epicoccoides*. *Science and Technology Journal of Agriculture and Rural Development* 12: 66-67.

Trang, T. T., Potter, K., Glen, M. and Mohammed, C. (2006) Molecular identification of fungi in rot types from logs in *Eucalyptus obliqua* forest in Tasmania. Poster presentation, PS1-97-0848. The 8th International Mycological Congress. Cairns, Australia.

Utomo, C., Werner, S., Niepold, F. and Deising H. B. (2005) Identification of *Ganoderma*, the causal agent of basal stem rot disease in oil palm using a molecular method. *Mycopathologia* 159: 159-170.

Van Niekerk, J. M., Groenewald, J. Z., Verkley, G. J. M. Fourie, P. H., Wingfield, M. J. and Crous, P. W. (2004) Systematic reappraisal of *Coniella* and *Pilidiella*, with specific reference to species occurring on *Eucalyptus* and *Vitis* in South Africa. *Mycological Research* 108: 283-303.

White, T. J., Bruns, T., Lee, S. and Taylor, T. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. (eds Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J.) 315-322 pp. Academic Press, San Diego, USA.

Williams, E. R., Matheson, A. C., Harwood, C.E. (2002) Experimental Design and Analysis for Tree Improvement, second edition. CSIRO Publishing.

Wingfield, M. J, Crous P. W. and Boden D. (1996) *Kirramyces destructans* sp. nov., a serious leaf pathogen of *Eucalyptus* in Indonesia. *South African Journal of Botany* 62: 325-327.

Yee, M. (2005) The ecology and habitat requirements of saproxylic beetles native to Tasmanian wet eucalypt forests: potential impacts of commercial forestry practices. PhD Thesis, University of Tasmania, Australia.

Yuan, Z. Q. (1996) Fungi and associated tree diseases in Melville Island, Northern Territory, Australia. *Australian Systematic Botany* 9: 337-360.

Yuan, Z. Q. (1999) Fungi Associated with Diseases Detected during Health Surveys of Eucalypt Plantations in Tasmania. Report to Forest and Wood Products Research and Development Corporation. School of Agricultural Science, University of Tasmania, Hobart, Australia. 110 pp.

Appendices

Appendix 1.1: Clones and landrace tested at each site.

Clone	Species[1]	Trial: Minh Duc (44 clones)	Song May (40 clones)	Bau Bang (10 clones)	Origin
101	*E tere*	X			Vietnam
201	*E. tere*	X	X		Vietnam
302	*E. cam*	X	X		Vietnam
350	N.a.		X		Chon Thanh
401	*E. tere*	X	X		Vietnam
402	*E. cam*	X	X		Vietnam
501	*E tere*		X		Chon Thanh
502	*E. tere*	X	X		Vietnam
601	*E. tere*	X	X		Vietnam
701	*E. tere*	X			Vietnam
902	*E. cam*	X			Vietnam
1001	*E cam*	X	X		Vietnam
1002	*E. cam*		X		Vietnam
1302	*E. cam*	X			Vietnam
1501	*E. cam*	X	X		Vietnam
1602	*E cam*	X	X		Vietnam
1902	*E. cam*		X		Chon Thanh
2002	*E. cam*	X			Vietnam
2202	*E. cam*		X		Chon Thanh
2502	*E. cam*	X			Vietnam
2702	*E. cam*	X			Vietnam
2802	*E. cam*	X			Vietnam
3302	*E. cam*		X		Chon Thanh
3402	*E. cam*	X	X		Vietnam
3502	*E. cam*		X		Chon Thanh
3602	*E cam*		X		Chon Thanh
3702	*E. cam*	X			Chon Thanh
3802	*E cam*	X	X		Vietnam
4102	*E. cam*	X	X		Vietnam
4202	*E. cam*	X			Chon Thanh
4402	*E. cam*	X			Vietnam
4502	*E. cam*	X			Vietnam
4602	*E cam*	X	X		Vietnam
4702	*E. cam*	X	X		Vietnam
4802	*E cam*	X	X		Vietnam
4902	*E. cam*		X		Chon Thanh
5002	*E. cam*		X		Vietnam
5102	*E. cam*	X	X		Vietnam
5202	*E. cam*	X	X		Vietnam
5302	*E. cam*		X		Vietnam
5402	*E. cam*	X	X		Vietnam
6402	*E. cam*	X			Chon Thanh
7301	N.a.	X			Song May
9107	*E. cam*	X	X	X	Song May
9410	*E. bra*	X	X		Vietnam

Appendix 1.1 (continued)

Clone	Species[1]	Trial: Minh Duc (44 clones)	Trial: Song May (40 clones)	Trial: Bau Bang (10 clones)	Origin
9495	*E. cam*	X	X	X	Song May
9498	*E. cam*	X	X	X	Song May
9501	*E. cam*		X	X	Song May
9518	*E. cam*	X	X	X	Song May
9901	*E. cam*		X		Chon Thanh
9902	*E. cam*	X			Vietnam
9903	*E. cam*	X			Chon Thanh
9904	*E. tere*	X	X		Song May
9905	*E. uxc*	X	X	X	China
9906	*E. uxc*			X	China
9907	*E. uxc*	X			PLPRC
9908	*N.a.*	X			Chon Thanh
9909	*Landrace*	X	X	X	Vietnam
9910	*E. uro*		X		SEFSPC
9911	*E tere*			X	SEFSPC
9912	*Hybrid*			X	PLPRC

X denotes clone tested at the site.

[1] *E. cam = E. camaldulensis, E. uro = E. urophylla, E. uxc = E. urophylla* x *camaldulensis* hybrid clone, *E. tere = E tereticornis, E. bra = E. brassiana,* N.a. = not available
seed = Landrace control.

Vietnam = originating from a province other than Chon Thanh or Song May
PLPRC = Phu Linh Paper Research Centre in Vietnam
SEFSPC = South Eastern Forest Science and Product Centre in Vietnam

Appendix 1.2: Classification of crown damage indices.

Class 1

Class 2

Class 3

Class 4

Appendix 1.2 (continued): Classification of crown damage indices.

	
Class 5	Class 6

Note: Class 1 equated to a nil or low level of premature crown defoliation or chlorosis resulting from fungal infection whereas class 6 represented total or almost total defoliation and dieback. The intermediate class 2-5 increased stepwise in severity relative to percent of crown damaged by fungal disease.

Appendix 1.3.1: Summary table of results of clonal eucalypt trials for each of the 3 sites; Minh Duc site for the assessment at 17 months after planting (44 clones); Song May site for the assessment at 29 months after planting (40 clones); Bau Bang site for the assessment at 29 months after planting (10 clones)

Clone	Species[1]	H (m)	Dgl (cm)	Survival (%)	CDI[2]	Fungal pathogen present
101	*E. tere*	4.2	3.5	71.9	1.7	*Coniella* sp.
201	*E. tere*	3.4	3.0	62.5	2.2	*C. eucalypti, Coniella* sp.
201	E. tere	6.1	4.8	44.0	2.6	Coniella sp.
302	*E. cam*	4.4	3.7	81.2	2.9	Not identified
302	E. cam	7.4	7.0	76.0	4.1	Kirramyces destructans
350	N.a.	7.4	6.6	60.0	2.0	Not identified
401	*E. tere*	3.0	2.8	75.0	4.5	Not identified
401	E. tere	6.6	6.4	76.0	3.2	Not identified
402	*E. cam*	4.6	3.6	46.9	2.6	*Coniella fragariae*
402	*E. cam*	8.1	7.6	84.0	2.6	*Coniella* sp.
501	*E. tere*	6.4	6.0	64.0	4.2	*Pestalotiopsis* sp.
502	*E. tere*	4.6	3.6	71.9	2.3	Not identified
502	*E. tere*	7.1	5.9	100.0	3.1	Not identified
601	*E. tere*	5.7	5.4	64.0	2.7	*Pestalotiopsis* sp.
701	*E. tere*	3.4	2.5	37.5	4.1	*Coniella fragariae*
902	*E. cam*	4.4	3.1	53.1	5.3	Not identified
1001	*E. cam*	5.0	4.4	75.0	1.1	Not identified
1001	*E. cam*	7.5	7.5	100.0	2.4	Not identified
1002	*E. cam*	7.0	6.3	76.0	4.6	Not identified
1302	*E. cam*	5.1	3.7	81.2	2.6	*Coniella* sp.
1501	*E. cam*	5.3	4.4	65.6	1.4	*Coniella* sp.
1501	*E. cam*	8.2	7.7	84.0	3.5	Not identified
1602	*E. cam*	5.0	4.3	71.6	3.0	Not identified
1602	*E. cam*	8.0	7.7	88.0	2.5	*Cryptosporiopsis eucalypti*
1902	*E. cam*	7.5	6.8	76.0	3.8	*Coniella* sp.
2002	*E. cam*	4.3	3.2	46.9	1.9	*Coniella* sp.
2202	*E. cam*	5.8	6.3	72.0	3.4	Not identified
2502	*E. cam*	4.7	4.0	81.2	3.0	Not identified
2702	*E. cam*	3.9	3.5	87.5	1.6	Not identified
2802	*E. cam*	4.5	3.4	84.4	2.5	*Coniella* sp.
3302	*E. cam*	6.7	6.0	84.0	2.5	*Coniella* sp.
3402	*E. cam*	5.2	3.8	78.1	1.3	*Cryptosporiopsis eucalypti, Coniella* sp.
3402	*E. cam*	7.0	5.9	88.0	4.2	Not identified
3502	*E. cam*	8.4	7.9	76.0	1.9	*Cryptosporiopsis eucalypti*
3602	*E. cam*	7.0	6.6	80.0	3.3	Not identified
3702	*E. cam*	4.5	4.0	71.9	1.6	Not identified
3802	*E. cam*	4.4	3.6	90.6	3.4	*Coniella* sp.
3802	*E. cam*	7.2	7.2	68.0	3.2	*Coniella* sp.
4102	*E. cam*	4.8	4.3	59.4	2.5	Not identified
4102	*E. cam*	7.3	7.3	72.0	2.6	*Coniella australiensis*
4202	*E. cam*	4.5	3.5	75.0	2.0	Not identified
4402	*E. cam*	5.0	4.5	90.6	2.0	*Coniella* sp.
4502	*E. cam*	4.1	3.3	71.9	1.7	Not identified

Appendix 1.3.1 (continued) : Summary table of results of clonal ecualypt trials; Minh Duc site for the assessment at 17 months after planting (44 clones); Song May site for the assessment at 29 months after planting (40 clones); Bau Bang site for the assessment at 29 months after planting (10 clones)

Clone	*Species1*	H (m)	Dgl (cm)	Survival (%)	CDI2	Fungal pathogen present
4602	*E. cam*	4.1	3.3	40.6	2.5	Not identified
4602	*E. cam*	7.4	6.4	84.0	2.7	*Cryptosporiopsis eucalypti, Coniella fragariae*
4702	*E. cam*	3.9	3.2	53.1	2.3	Not identified
4702	*E. cam*	7.0	6.7	80.0	2.2	Not identified
4802	*E. cam*	4.0	3.1	71.9	4.7	Not identified
4802	*E. cam*	6.3	6.4	80.0	4.6	*Cylindrocladium quinqueseptatum*
4902	*E. cam*	6.5	5.4	64.0	3.5	Not identified
5002	*E. cam*	7.2	6.1	84.0	2.1	*Cylindrocladium quinqueseptatum*
5102	*E. cam*	4.8	3.9	75.0	1.8	*Coniella* sp.
5102	*E. cam*	7.9	7.2	68.0	3.3	*Microsphaeropsis globulosa*
5202	*E. cam*	4.4	3.4	81.2	4.4	*Coniella* sp.
5202	*E. cam*	6.6	5.9	80.0	2.4	Not identified
5302	*E. cam*	6.1	5.5	80.0	3.2	*Pestalotiopsis* sp., *Coniella fragariae*, gall
5402	*E. cam*	2.9	2.1	31.2	1.7	*Coniella* sp.
5402	*E. cam*	6.0	5.4	24.0	4.1	*Cylindrocladium quinqueseptatum*
6402	*E. cam*	4.6	3.7	53.1	3.8	*Coniella* sp.
7301	N.a.	5.2	4.1	78.1	1.8	*Coniella* sp.
9107	*E. cam*	4.8	3.7	71.9	2.8	*Coniella fragariae*, gall
9107	*E. cam*	7.9	7.8	96.0	2.5	*Coniella australiensis*, gall
9107	*E. cam*	8.6	6.3	90.0	3.7	*Coniella* sp., *Microsphaeropsis globulosa*
9410	*E. bra*	4.7	4.1	62.5	1.9	Not identified
9410	*E. bra*	7.9	7.2	92.0	1.4	*Pestalotiopsis* sp.
9495	*E. cam*	5.1	4.5	84.4	1.7	*Coniella* sp.
9495	*E. cam*	8.8	8.6	100.0	1.4	*Coniella fragariae, Pestalotiopsis* sp.
9495	*E. cam*	10.1	7.5	98.8	1.2	*Microsphaeropsis globulosa, Coniella* sp.
9498	*E. cam*	5.2	4.1	84.4	2.2	*Coniella fragariae*
9498	*E. cam*	8.0	7.8	84.0	2.6	Not identified
9498	*E. cam*	8.1	6.0	98.8	4.6	*Coniella australiensis, Microsphaeropsis globulosa*
9501	*E. cam*	6.1	5.2	40.0	3.4	*Coniella* sp.
9501	*E. cam*	6.3	4.5	71.2	3.3	*Coniella fragariae*
9518	*E. cam*	5.6	4.6	87.5	1.3	Not identified
9518	*E. cam*	8.4	8.1	88.0	1.5	*Coniella* sp., *Cryptosporiopsis eucalypti*
9518	*E. cam*	9.9	7.2	92.5	1.3	*Coniella fragariae*
9901	*E. cam*	7.0	6.6	92.0	2.6	*Pestalotiopsis* sp.
9902	*E. cam*	5.4	4.5	81.2	1.6	*Coniella* sp.
9903	*E. cam*	5.4	4.7	90.6	1.2	*Coniella fragariae*

Appendix 1.3.1 (continued): Summary table of results of clonal ecualypt trials; Minh Duc site for the assessment at 17 months after planting (44 clones); Song May site for the assessment at 29 months after planting (40 clones); Bau Bang site for the assessment at 29 months after planting (10 clones)

	Species1	H (m)	Dgl (cm)	Survival (%)	CDI2	Fungal pathogen present
9904	*E. tere*	4.4	3.6	71.9	1.4	Not identified
9904	*E. tere*	6.7	5.7	88.0	2.8	Not identified
9905	*E. uxc*	5.2	4.6	68.8	2.6	*Coniella fragariae*
9905	*E. uxc*	7.4	7.9	100.0	5.8	Not identified
9905	*E. uxc*	7.4	7.3	98.8	6.0	*Kirramyces destructans*
9906	*E. uxc*	8.3	8.1	96.2	6.0	*Kirramyces destructans*
9907	*E. uxc*	3.8	3.5	43.8	4.2	Not identified
9908	N.a.	4.6	4.3	68.8	4.3	*Coniella* sp., *Cryptosporiopsis eucalypti*
9909	Landrace	4.7	4.1	75.0	2.1	Not identified
9909	Landrace	6.5	5.6	44.0	1.9	*Kirramyces destructans*
9909	Landrace	7.7	7.3	62.5	1.6	*Coniella fragariae, Kirramyces destructans*
9910	*E. uro*	6.5	6.5	52.0	5.4	Not identified
9911	*E. tere*	7.3	5.9	88.8	2.3	*Coniella australiensis*
9912	Hybrid	8.1	6.6	93.8	2.7	Not identified
Grand mean		4.5	3.7	69	2.5	
F-probability		0.001	0.001	0.001	0.001	
		0.001	0.001	0.001	0.001	
		0.001	0.001	0.001	0.001	
S.e.d mean		0.33	0.36	11.93	0.46	
		0.47	0.45	13.65	0.49	
		0.5	0.31	6.58	0.22	

[1] *E. tere = E. tereticornis, E. cam = E. camaldulensis, E. bra = E. brassiana, , E. uro = E. urophylla E. uxc = E. urophylla* x *camaldulensis* hybrid clone, seed = Landrace control, N.a. = not available

[2] CDI = Crown damage index; S.e.d mean = standard error of difference of mean

Appendix 1.3.2: Summary table of estimated means for clones in combined site analysis.

Clone	Species[1]	Height (m)	Survival (%)	Crown damage index
101	*E. tere*	6.3	79.8	2.2
201	*E. tere*	5.5	61.2	2.6
302	*E. cam*	6.6	86.4	3.2
350	N.a.	6.7	68.0	1.9
401	*E. tere*	5.5	80.2	3.4
402	*E cam*	7.0	73.5	3.2
501	*E tere*	5 7	72.0	4.2
502	*E tere*	6 5	95.5	3.2
601	*E. tere*	5.2	58.7	2.8
701	*E. tere*	5.5	45.4	4.6
902	*E. cam*	6.5	67.3	5.2
1001	*E. cam*	7.0	92.3	2.5
1002	*E. cam*	6.3	85.0	3.9
1302	*E. cam*	7.2	89.1	3.1
1501	*E cam*	7.4	79.7	2.6
1602	*E cam*	7.2	83.0	3.1
1902	*E. cam*	6.8	84.0	3.7
2002	*E. cam*	6.4	54.8	2.4
2202	*E. cam*	5.1	80.0	3.3
2502	*E. cam*	6.8	89.1	3.4
2702	*E. cam*	6.0	87.6	2.7
2802	*E. cam*	6.6	92.3	3.0
3302	*E. cam*	6.0	92.0	2.4
3402	*E. cam*	6.8	91.0	3.0
3502	*E. cam*	7.7	85.0	2.8
3602	*E. cam*	6.3	90.2	3.0
3702	*E cam*	6.6	79.8	2.1
3802	*E. cam*	6.5	86.7	3.3
4102	*E. cam*	6.8	69.5	3.0
4202	*E. cam*	6.6	82.9	2.5
4402	*E. cam*	7.1	76.6	2.9
4502	*E. cam*	6.2	79.8	3.0
4602	*E. cam*	6.4	73.4	2.8
4702	*E. cam*	6.2	80.5	2.0
4802	*E. cam*	5.8	83.9	4.9
4902	*E cam*	5.8	72.0	3.4
5002	*E. cam*	6.5	92.0	2.0
5102	*E. cam*	7.0	79.6	2.8
5202	*E. cam*	6.2	83.6	3.4
5302	*E cam*	5.4	88.0	3.1
5402	*E. cam*	5.1	42.0	3.4
6402	*E. cam*	6.7	61.0	4.3
7301	N.a.	7.3	86.0	2.3
9107	*E. cam*	7.1	84.8	2.9
9410	*E. bra*	7.0	58.2	1.9
9495	*E. cam*	8.0	96.0	1.5
9498	*E. cam*	7.1	78.3	2.9
9501	*E cam*	5.1	50.9	3.0
9518	*E. cam*	8.0	95.6	1 8

Appendix 1.3.2 (continued) Summary table of estimated means for clones in combined site analysis.

Clone	Species[1]	Height (m)	Survival (%)	Crown damage index
9901	*E. cam*	6.3	100.0	2.6
9902	*E. cam*	7.5	93.0	2.0
9903	*E cam*	7.5	98.5	1.7
9904	*E. tere*	6.3	87.9	2.3
9905	*E. uxc*	6.7	68.2	4.2
9906	*E. uxc*	6.9	81.7	5.4
9907	*E. uxc*	5.9	51.7	4.6
9908	N.a.	6.7	76.7	4.7
9909	Landrace	6.3	75.7	1.8
9910	*E. uro*	5.8	60.0	5.3
9911	*E tere*	5.9	74.3	1.7
9912	Hybrid	6.7	82.7	1.7

[1] *E cam* = *E. camaldulensis*, *E uro* = *E. urophylla*, *E uxc* = *E. urophylla* x *camaldulensis* hybrid clone, *E. tere* = *E. tereticornis*, *E. bra* = *E. brassiana*, N.a. = not available
seed = Landrace control.

Appendix 1.3.3: Pooled residual mean square analysis for combined site analysis.

Variate	d.f	s.s	m.s.	v.r.	F-probability
Height					
Site	2	195.0908	97.5454	563.87	<.001
Clone	60	48.0306	0.8005	4.63	<.001
Site.clone	31	5.3628	0.173	1.76	<.001
Pooled residual	484		0.098		
Survival					
Site	2	3432.4	1716.2	6.98	0.018
Clone	60	17140.7	285.7	1.16	0.819
Site.clone	59	14505.8	245.9	3.97	<.001
Pooled residual	484		61.94		
Crown damage index					
Site	2	8.9033	4.4517	6.06	0.006
Clone	60	95.0974	1.585	2.16	0.011
Site.clone	31	22.7606	0.7342	8.85	<0.001
Pooled residual	484		0.088		

d.f. = degrees of freedom, s.s. = sum of squares, m.s. = mean square, v.r. = variance ratio

Appendix 1.4: Disease symptoms and signs observed.

Figure A1.4.1: *Coniella fragariae* on *E. camaldulensis.*

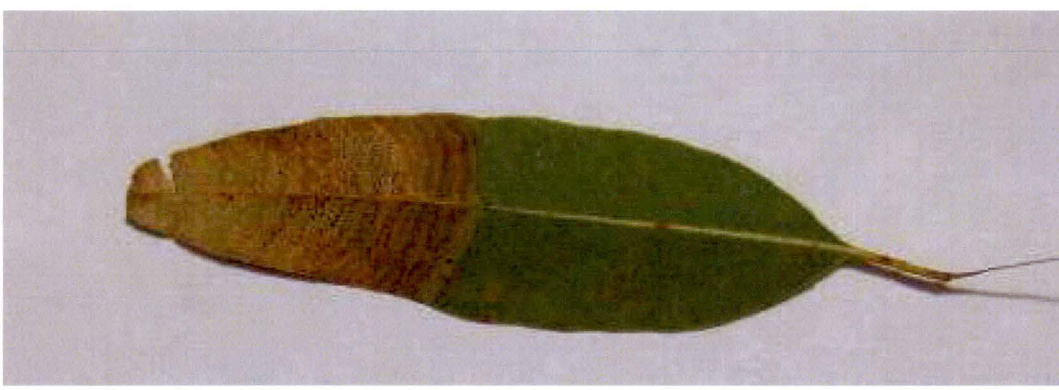


Figure A1.4.2: *Coniella australiensis* on *E. tereticornis.*

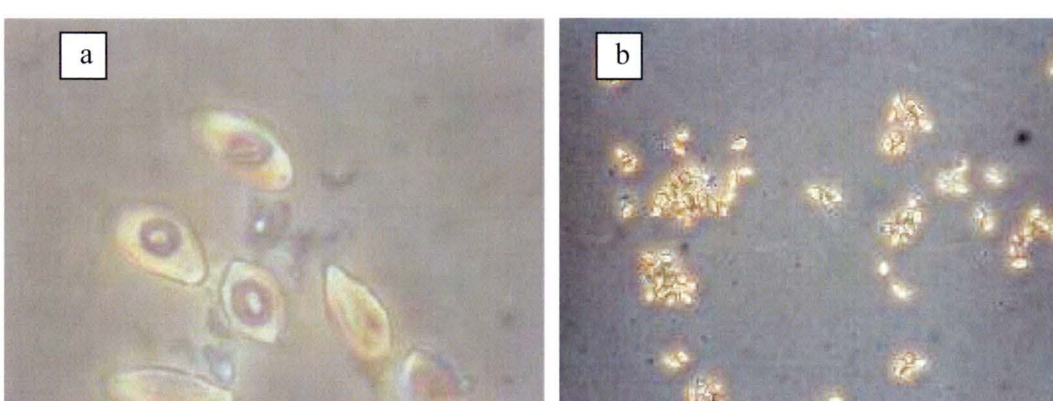


Figure A1.4.3: (a) Conidia of *Coniella fragariae* (b) Conidia of *Coniella australiensis.*

Figure A1.4.4: *E. camaldulensis* infected by *Cryptosporiopsis eucalypti.*

Figure A1.4.5: *E. urophylla* associated with *Cryptosporiopsis eucalypti*
(Photo: P.Q. Thu).

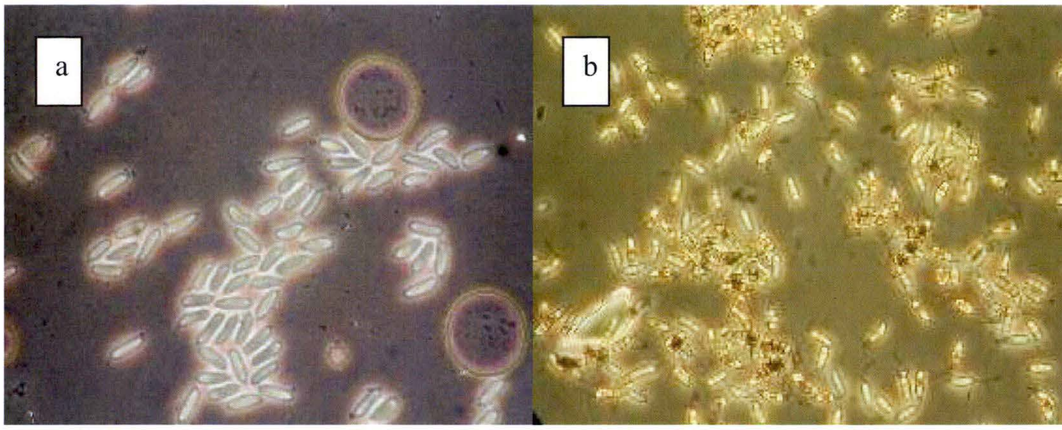


Figure A1.4.6: Conidia of (a) *Cryptosporiopsis eucalypti* and (b) *Pestalotiopsis* sp. (Photo: P.Q. Thu).

Figure A1.4.8: *E. urophylla* infected by *Ralstonia solanasearum* in Hoa Binh (Photo: P.Q. Thu).

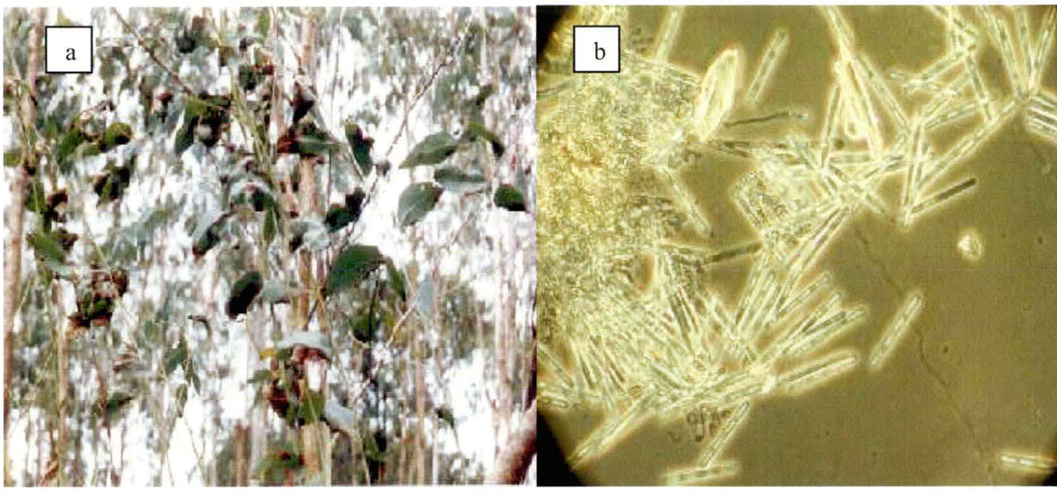


Figure A1.4.9: (a) *E. camaldulensis* associated with *Cylindrocladium* in Song May (Photo: P.Q. Thu) and (b) conidia of *Cylindrocladium quinqueseptatum.*

Figure A1.4.11: *Kirramyces destructans* associated with clone-9905 in Dai Lai.

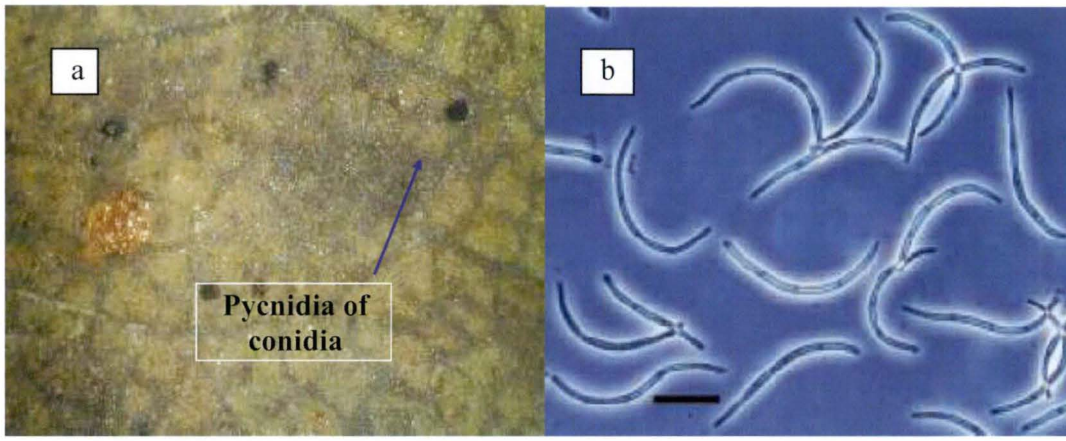


Figure A1.4.12: (a) *Kirramyces destructans* on the lower side of *E. camaldulensis* leaf (b) Typically three-septate, sinuous conidia of *P. destructans*, bar = 25μm (Photo: Ken Old).

Figure A1.4.13: *E. camaldulensis* infected by *Kirramyces destructans* in Bau Bang.

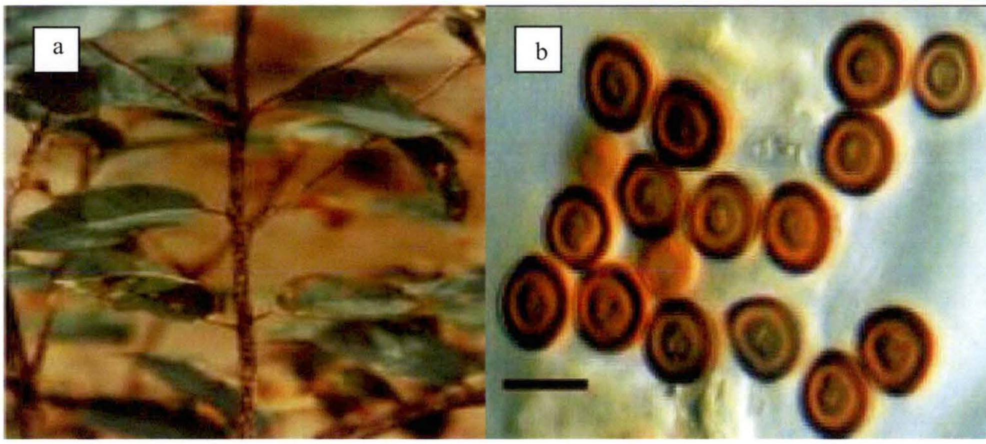


Figure A1.4.14: *Microsphaeropsis globulosa* (a) and its conidia (b) (bar = 0.7µm) infected on *E. grandis* in Sri Lanka (Photo: Ken Old).

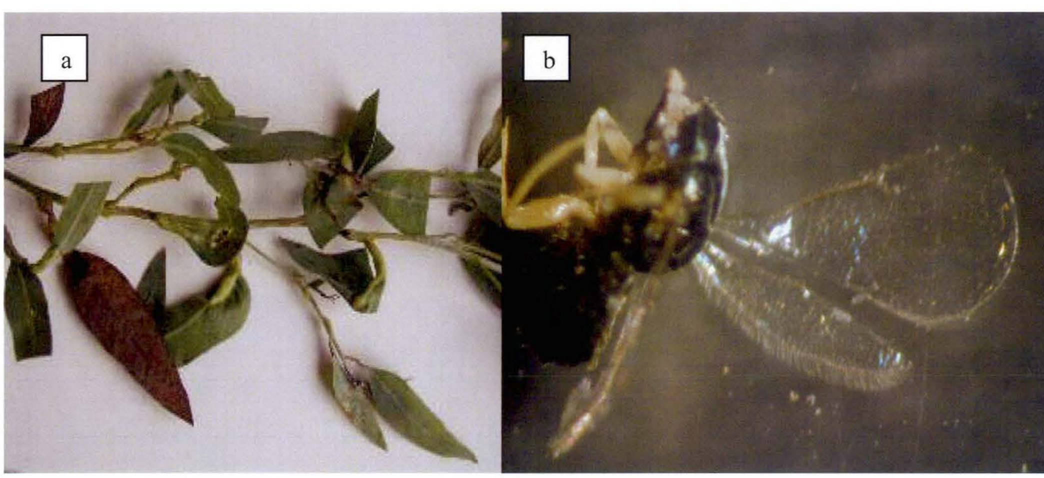


Figure A1.4.15: (a) Galls on *E. camaldulensis* and (b) *Leptocybe invasa* associated with gall.

Appendix 2.1: Sporocarps and collection locations.

Herbarium code	Collection location
E7501	Gould's country, N.E. Tasmania - thinning and pruning trial
E7503	Gould's country, N.E. Tasmania - thinning and pruning trial
E7505	Gould's country, N.E. Tasmania - thinning and pruning trial
E7506	Gould's country, N E. Tasmania - thinning and pruning trial
E7508	Gould's country, N.E. Tasmania - thinning and pruning trial
E7510	Gould's country, N.E. Tasmania - thinning and pruning trial
E7511	Gould's country, N.E. Tasmania - thinning and pruning trial
E7514	Gould's country, N.E. Tasmania
E7516	Gould's country, N.E. Tasmania
E7517	Gould's country, N.E. Tasmania
E7518	Gould's country, N.E. Tasmania
E7519	Gould's country, N.E. Tasmania
E7520	Gould's country, N.E. Tasmania
E7526	Gould's country, N.E. Tasmania
E7528	"Lumeah" Adventure Bay, S Bruny
E7529	"Lumeah" Adventure Bay, S Bruny
E7534	Mavista walk, S Bruny (5km from Adventure Bay)
E7535	Gould's country, N.E. Tasmania
E7536	(2yo regen) Gould's country, N.E. Tasmania
E7537	(2yo regen) Gould's country, N.E. Tasmania
E7538	Gould's country, N.E. Tasmania
E7539	Gould's country, N.E. Tasmania
E7540	Gould's country, N.E. Tasmania
E7541	(2yo regen) Gould's country, N.E. Tasmania
E7542	Gould's country, N.E. Tasmania
E7543	(2yo regen) Gould's country, N.E. Tasmania
E7544	(2yo regen) Gould's country, N.E. Tasmania
E7545	(2yo regen) Gould’s country, N.E. Tasmania
E7546	(2yo regen) Gould’s country, N.E. Tasmania
E7547	(2yo regen) Gould’s country, N.E. Tasmania
E7548	(2yo regen) Gould’s country, N.E. Tasmania
E7549	(2yo regen) Gould’s country, N.E. Tasmania
E7550	Gould’s country, N.E. Tasmania
E7551	Gould’s country, N.E. Tasmania
E7552	Gould’s country, N.E. Tasmania
E7553	Gould’s country, N.E. Tasmania
E7554	Gould’s country, N.E. Tasmania
E7555	Gould’s country, N.E. Tasmania
E7556	Gould’s country, N.E. Tasmania
E7557	Gould’s country, N.E. Tasmania
E7558	Gould’s country, N.E. Tasmania
E7559	Gould’s country, N.E. Tasmania
E7560	Gould’s country, N.E. Tasmania
E7561	Gould’s country, N.E. Tasmania
E7562	(2yo regen) Gould’s country, N.E. Tasmania
E7563	(2yo regen) Gould’s country, N.E. Tasmania
E7564	Gould’s country, N.E. Tasmania
E7565	Gould’s country, N.E. Tasmania
E7566	Gould’s country, N.E. Tasmania
E7567	Gould’s country, N.E. Tasmania
E7568	Gould’s country, N.E. Tasmania
E7569	Gould’s country, N.E. Tasmania
E7570	Gould’s country, N.E. Tasmania

Appendix 2.1 (continued)

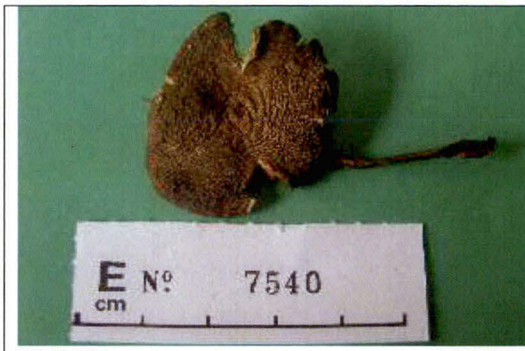
Herbarium code	Collection location
E7571	Gould's country, N.E. Tasmania
E7572	(2yo regen) Gould's country, N.E. Tasmania
E7573	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7574	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7575	Gowrie Park, O'Neill's Creek, N.W Tasmania
E7576	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7577	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7578	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7579	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7580	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7581	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7582	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7583	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7584	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7585	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7586	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7587	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7588	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7589	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7590	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7591	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7592	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7593	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7594	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7595	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7596	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7597	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7598	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7599	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7600	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7601	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7602	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7603	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7604	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7605	Gowrie Park, O'Neill's Creek, N.W. Tasmania
E7611	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7612	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7613	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7614	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7615	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7616	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7617	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7618	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7619	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7620	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
E7621	Aggregated Retention Coupe WR008I, Warra LTER, S. Tasmania
15948B1	Darwin, Northern Territory
15948B2	Darwin, Northern Territory
15948B3	Darwin, Northern Territory
16313A1	Darwin, Northern Territory
16313A2	Darwin, Northern Territory
16313A3	Darwin, Northern Territory

Appendix 2.1 (continued)

Herbarium code	Collection location
16452D1	*Carpentaria acuminata*, Northern Territory
16452D2	*Carpentaria acuminata*, Northern Territory
16452D3	*Carpentaria acuminata*, Northern Territory
16453C1	*Dypsis lutescens*, Northern Territory
16453C2	*Dypsis lutescens*, Northern Territory
16453C3	*Dypsis lutescens*, Northern Territory
16722A	*Casuarina equisetifolia*, Northern Territory
167311B	*Dypsis lutescens*, Northern Territory
16732C	*Carpentaria acuminata*, Northern Territory

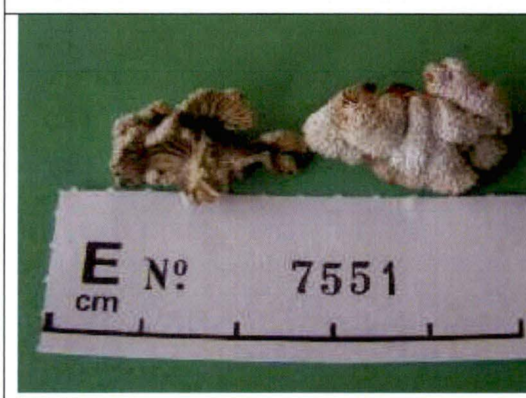
Appendix 2.2: Sporocarp images and isolates.

Appendix 2.2.1: Sporocarps used in section 2.



E7540

E7548

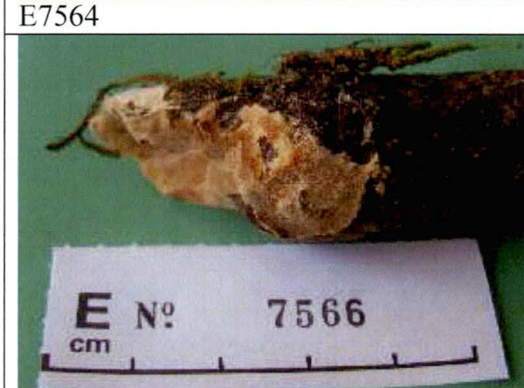


E7551

E7552

E7564

E7565



E7566

E7568

E7569

E7570


E7575


E7576


E7577


E7578


E7580


E7581


E7586


E7588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E7590


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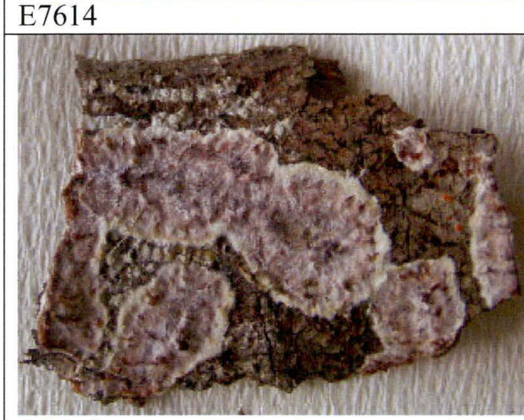
E7601

E7608

E7612

E7614

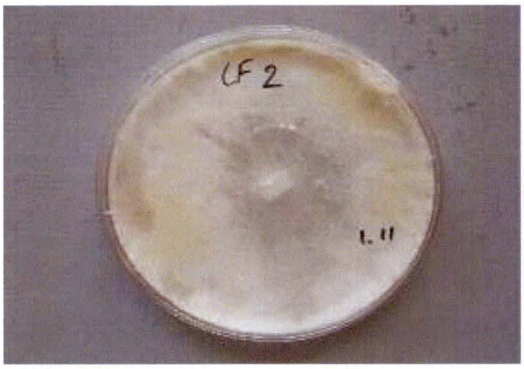
E7615



E7616

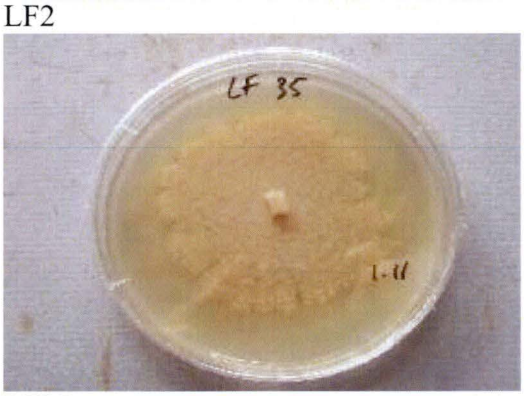
E7618

Appendix 2.2.2: Isolates (LF) obtained from decayed log.

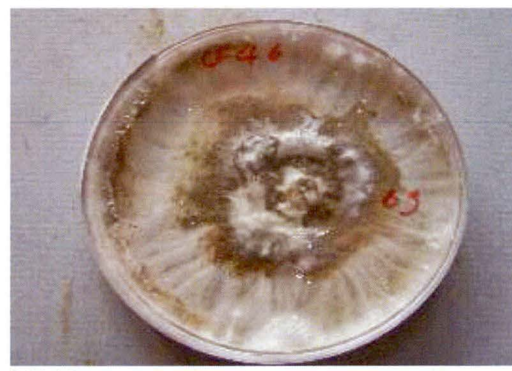


LF2

LF5



LF35



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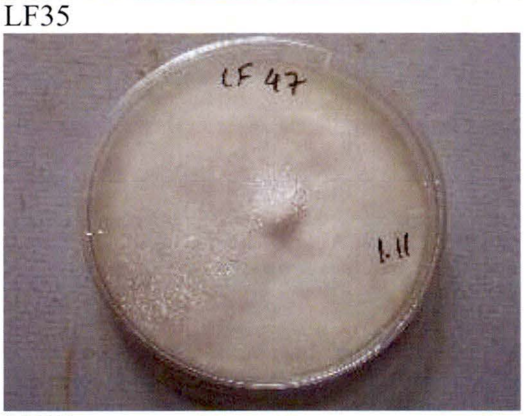
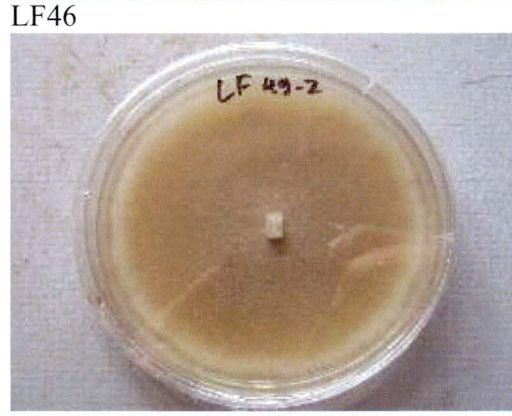


LF47



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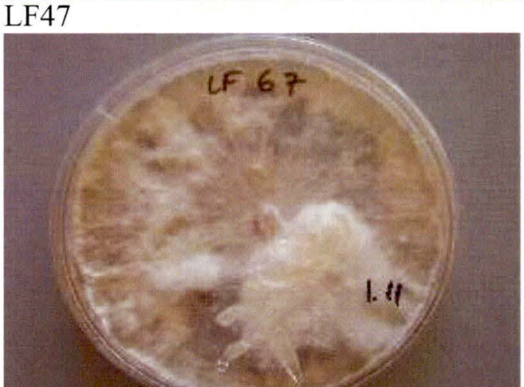
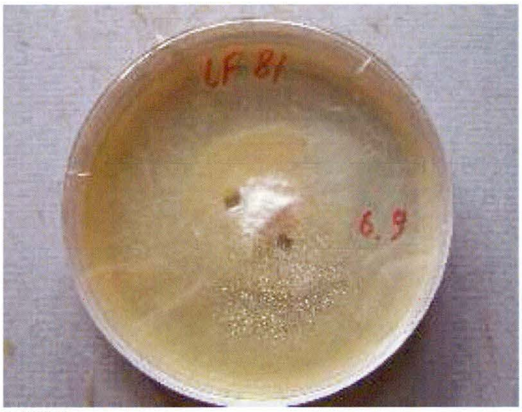


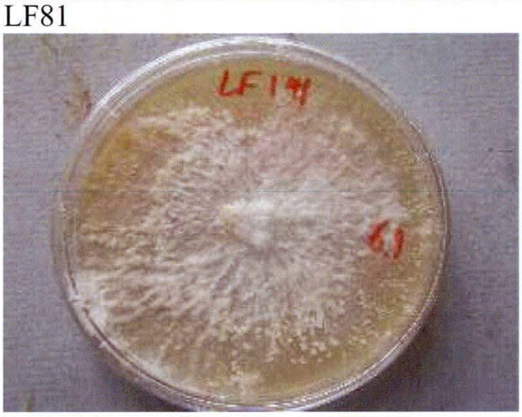
LF67

LF77

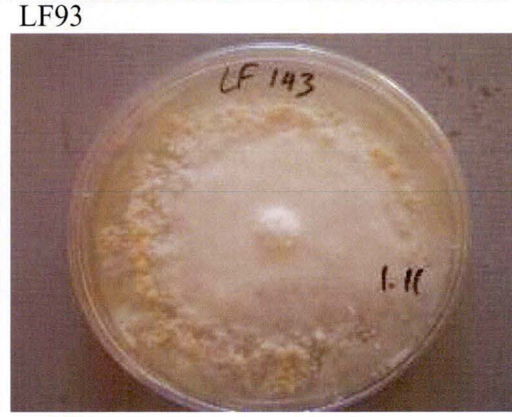


LF81

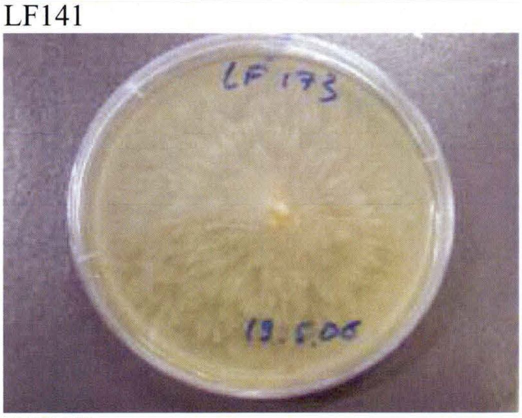
LF93



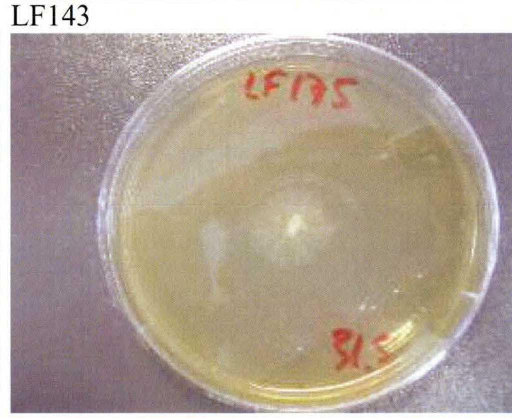
LF141



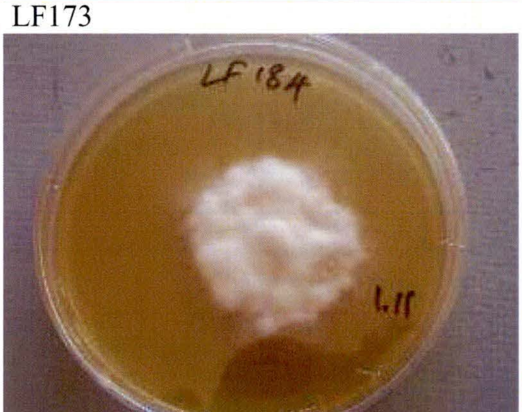
LF143



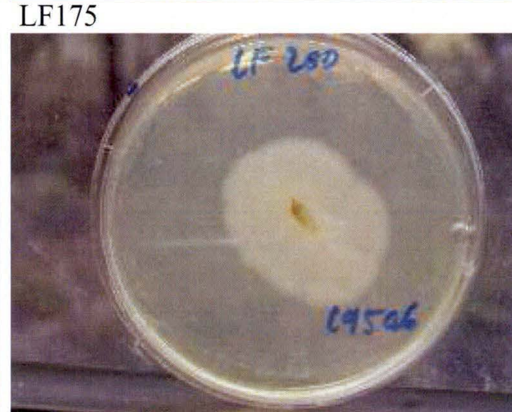
LF173



LF175

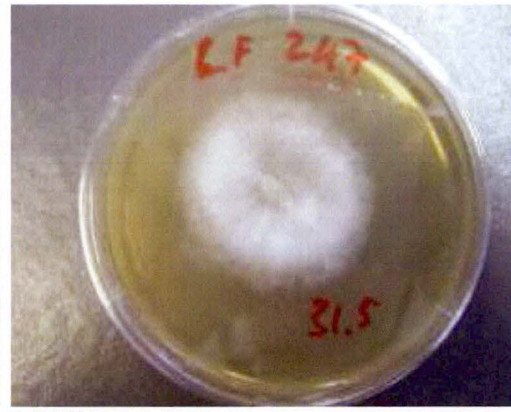


LF184



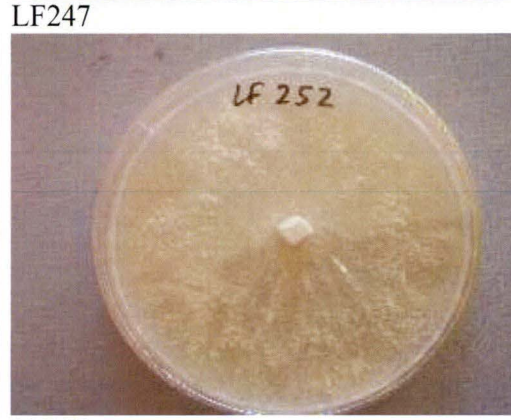
LF200


LF234

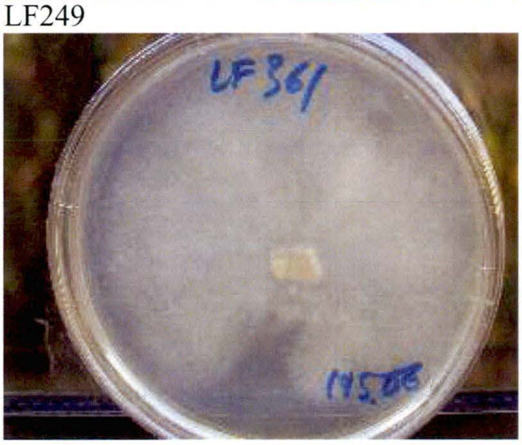


LF247


LF249



LF252



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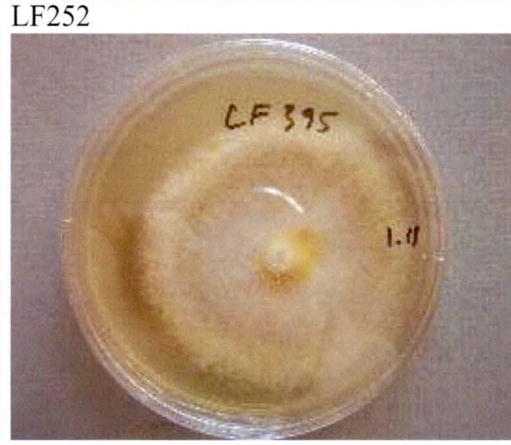
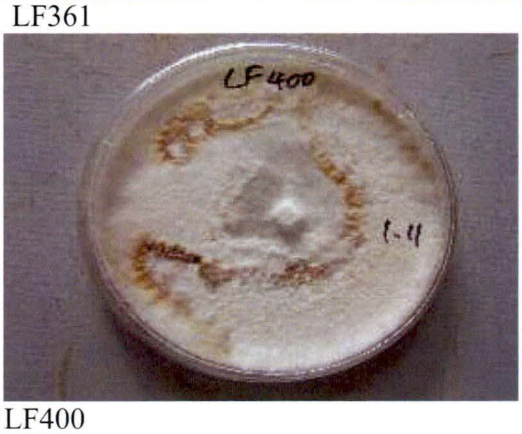
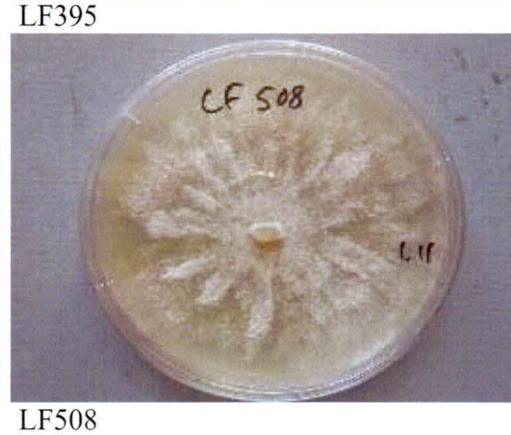
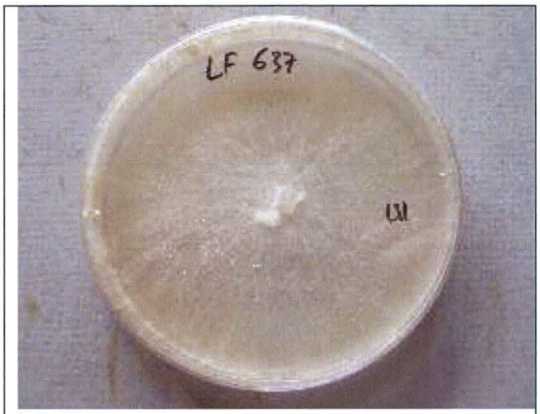


LF395



LF400


LF508



LF637

Appendix 2.3: Rotten wood samples.


1.EDL1.1


2. EDL1.6

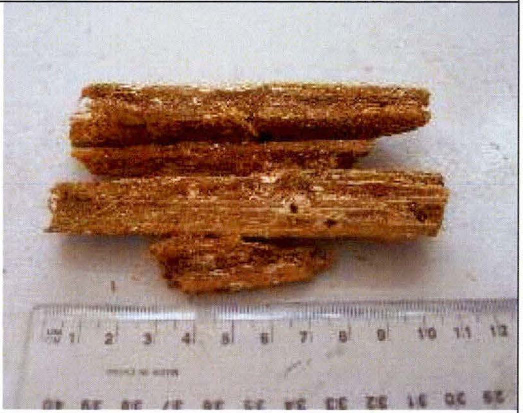

3. EDS1.1


4. EDS1.5

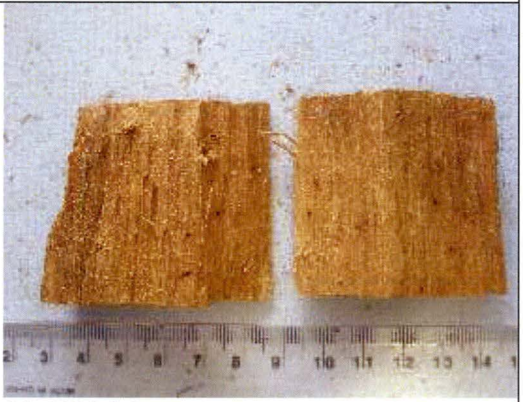

5. EDS2.5


6. ERDS2.3


7. EDL2.1


8. ERDL2.1


9. EDL2.6


10. EDL3.3


11. ERDS3.1


12. SWDL2.5


13. SWDS2.1


14. SWDS3.3


15. SWDS3.32


16. HL3.5


17. HC2.7


18. MRDL2.6


19. MRDS1.4


20. WRDL2.5

21. WRDS2.3

22. WRQ1.14

23. WRQ1.3b

24. SWQ1.4

25. SWQ2

26. SWQ3.13

Appendix 2.4: Maximum likelihood dendrograms used to determine the most likely placement of the unknown fungus.

Figure A2.4.1: Maximum likelihood tree from analysis of ITS sequences of isolate P0835. The outgroup *Trametes hirsuta* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

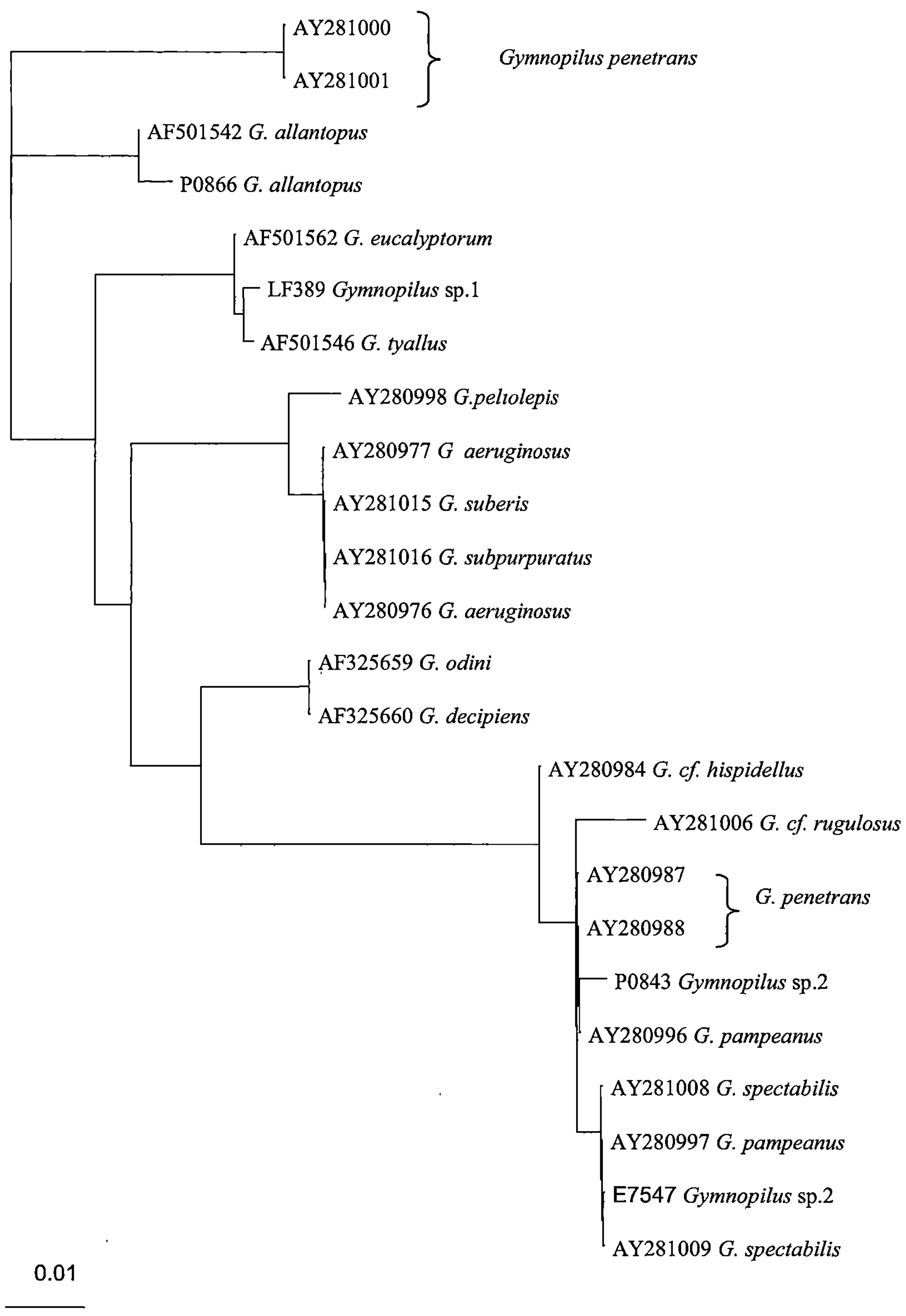


Figure A2.4.2: Maximum likelihood tree from analysis of ITS sequences of sporocarp E7547 and isolates P0843, P0866 and LF389. The outgroups *Gymnopilus penetrans* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

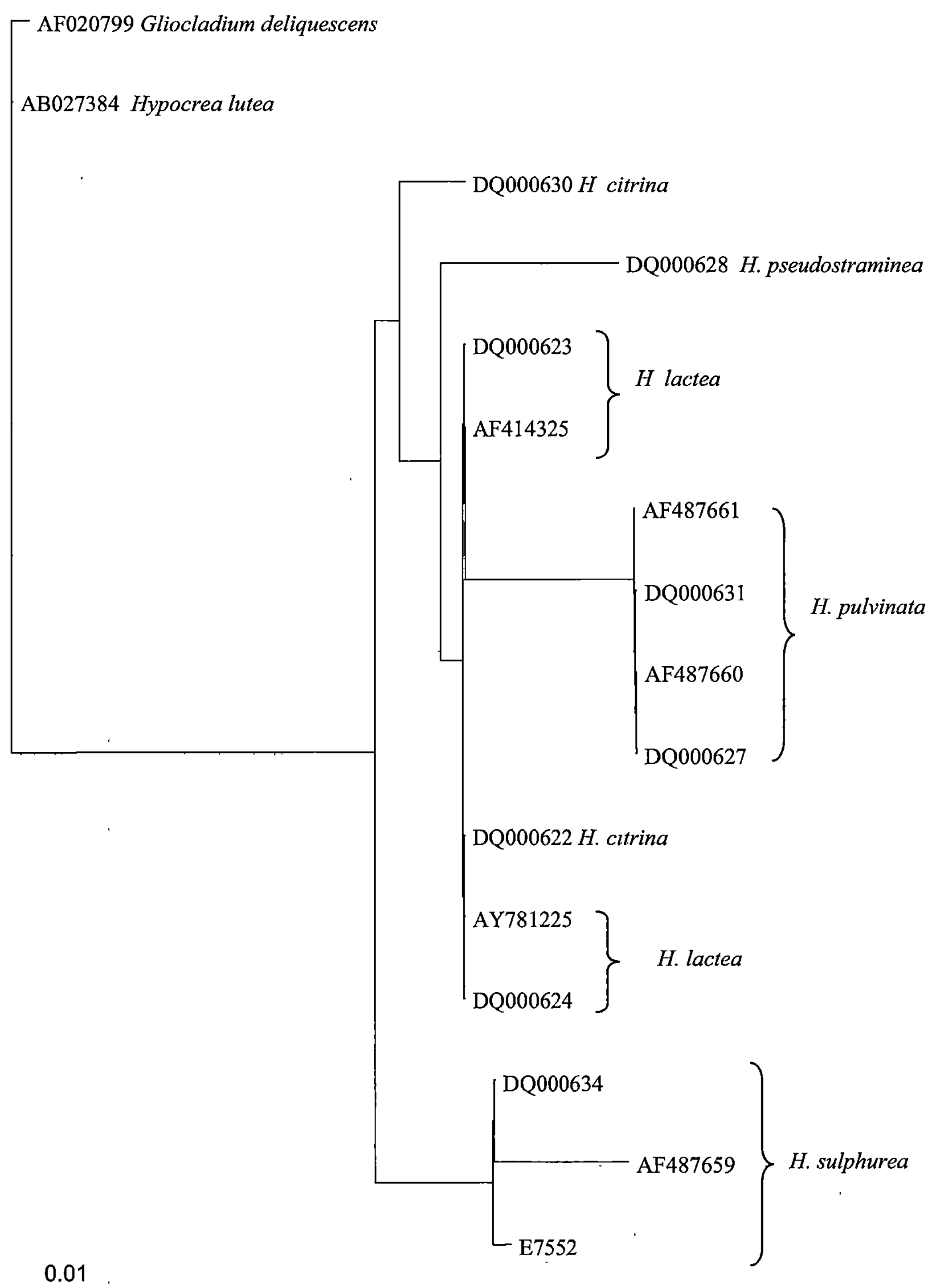


Figure A2.4.3: Maximum likelihood tree from analysis of ITS sequences of sporocarp E7552. The outgroup *Gliocladium deliquescens* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

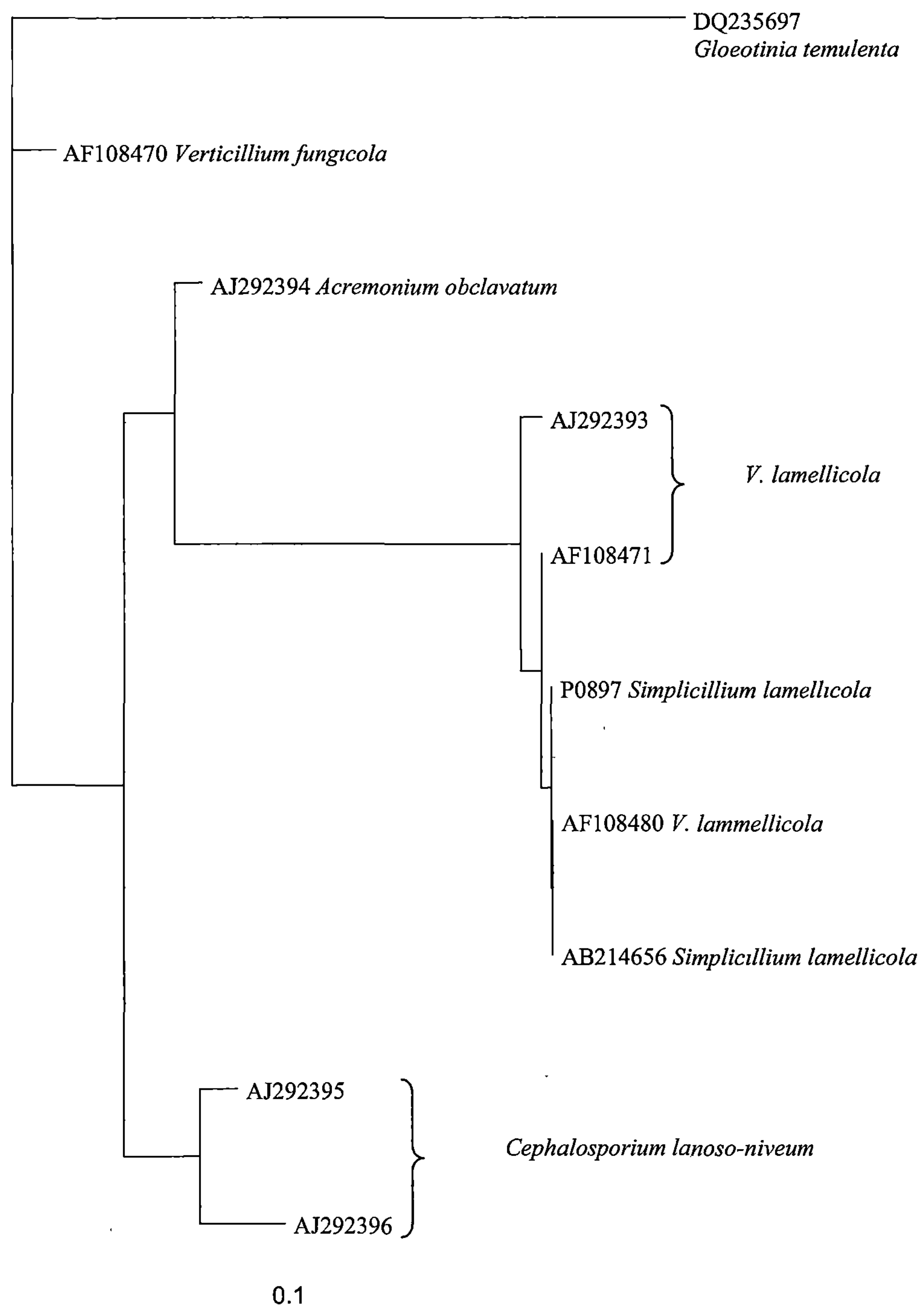


Figure A2.4.4: Maximum likelihood tree from analysis of ITS sequences of isolate P0897. The outgroup *Gloeotinia temulenta* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 10%.

Note: *Verticillium lamellicola* is a synonym of *Simplicillium lamellicola*.

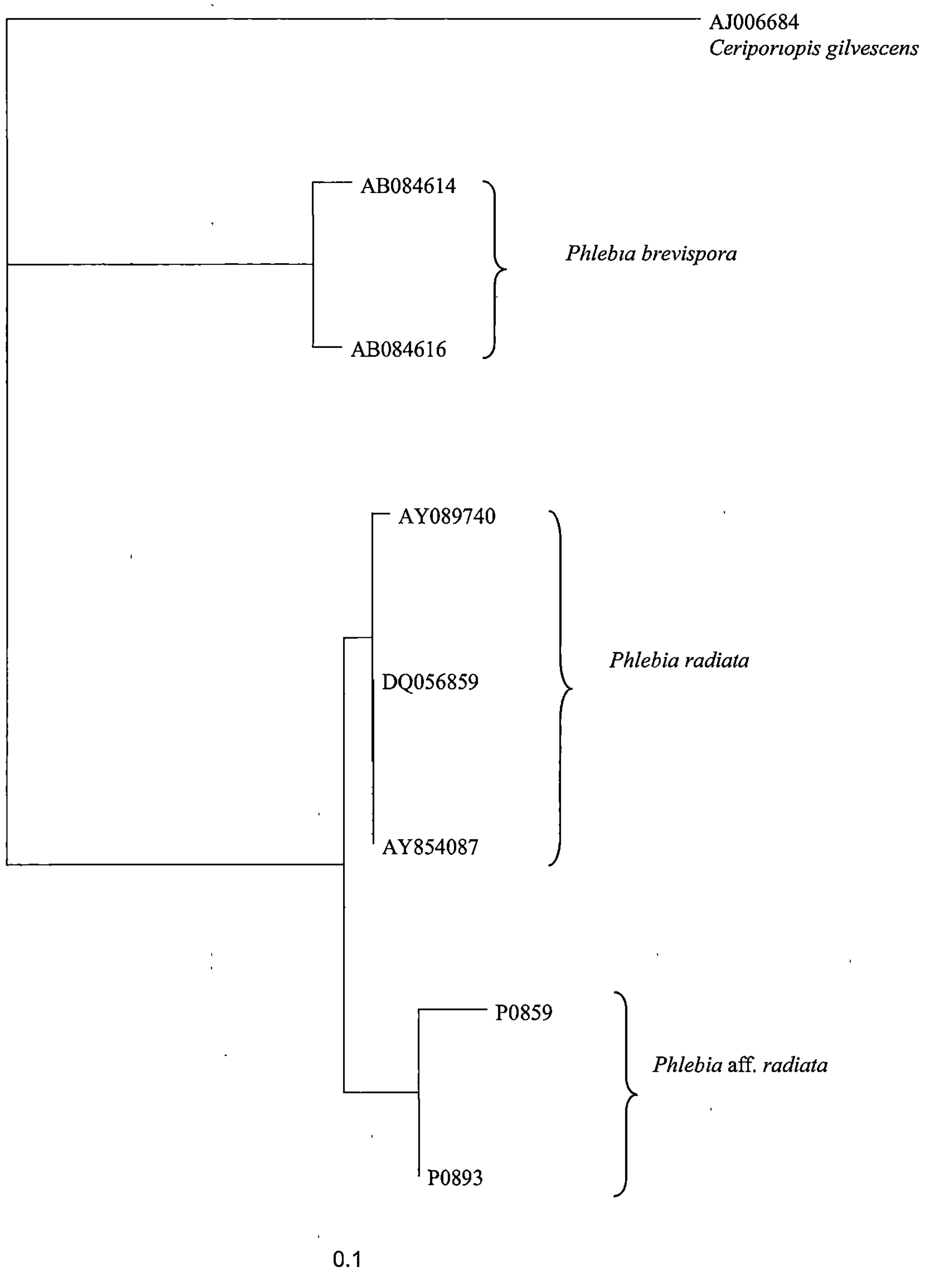


Figure A2.4.5: Maximum likelihood tree from analysis of ITS sequences of isolates P0859 and P0893. The outgroup *Ceriporiopis gilvescens* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 10%.

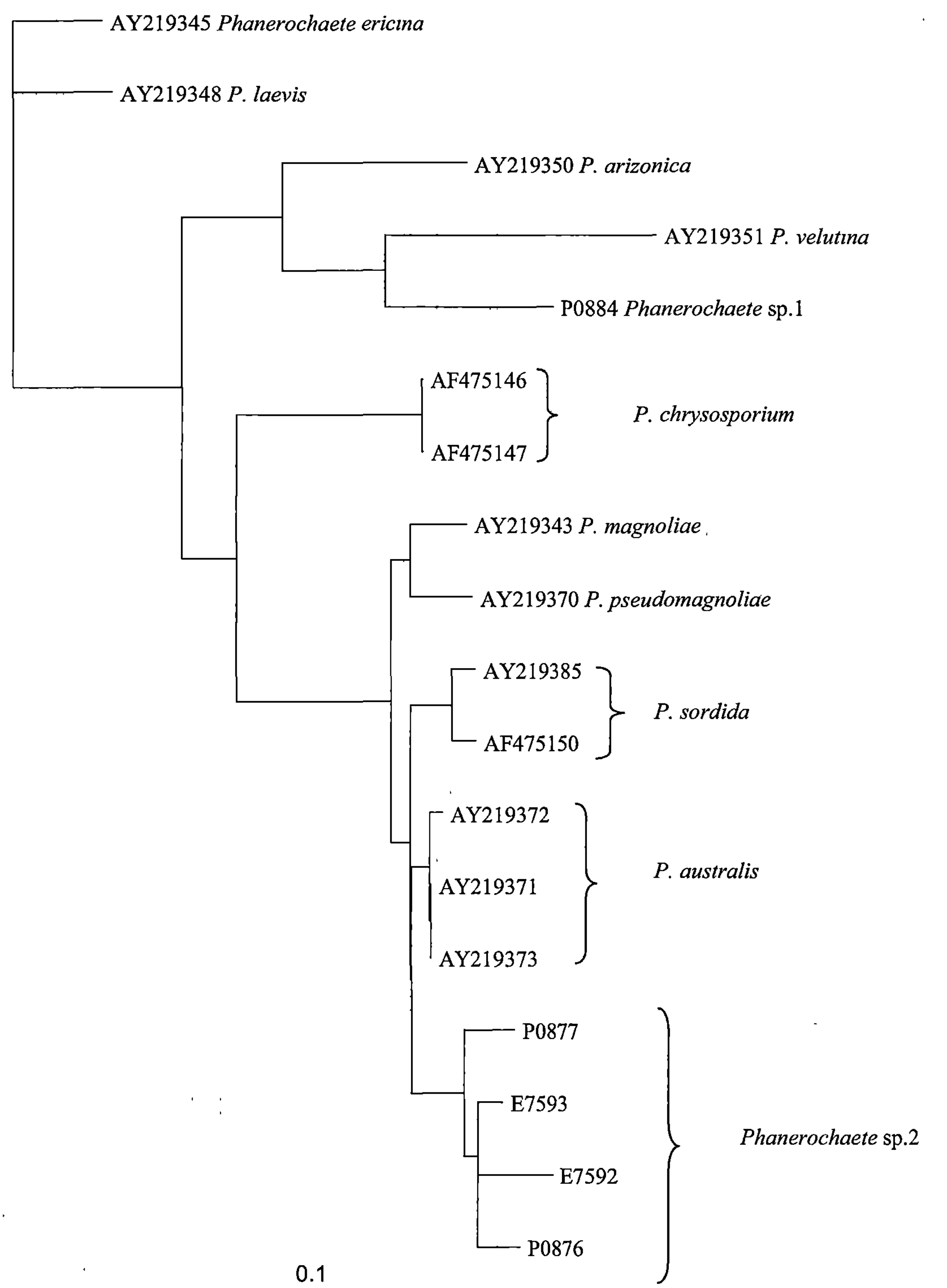


Figure A2.4.6: Maximum likelihood tree from analysis of ITS sequences of sporocarps E7592, E7593 and isolates P0876 and P0877. The outgroup *Phanerochaete ericina* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 10%.

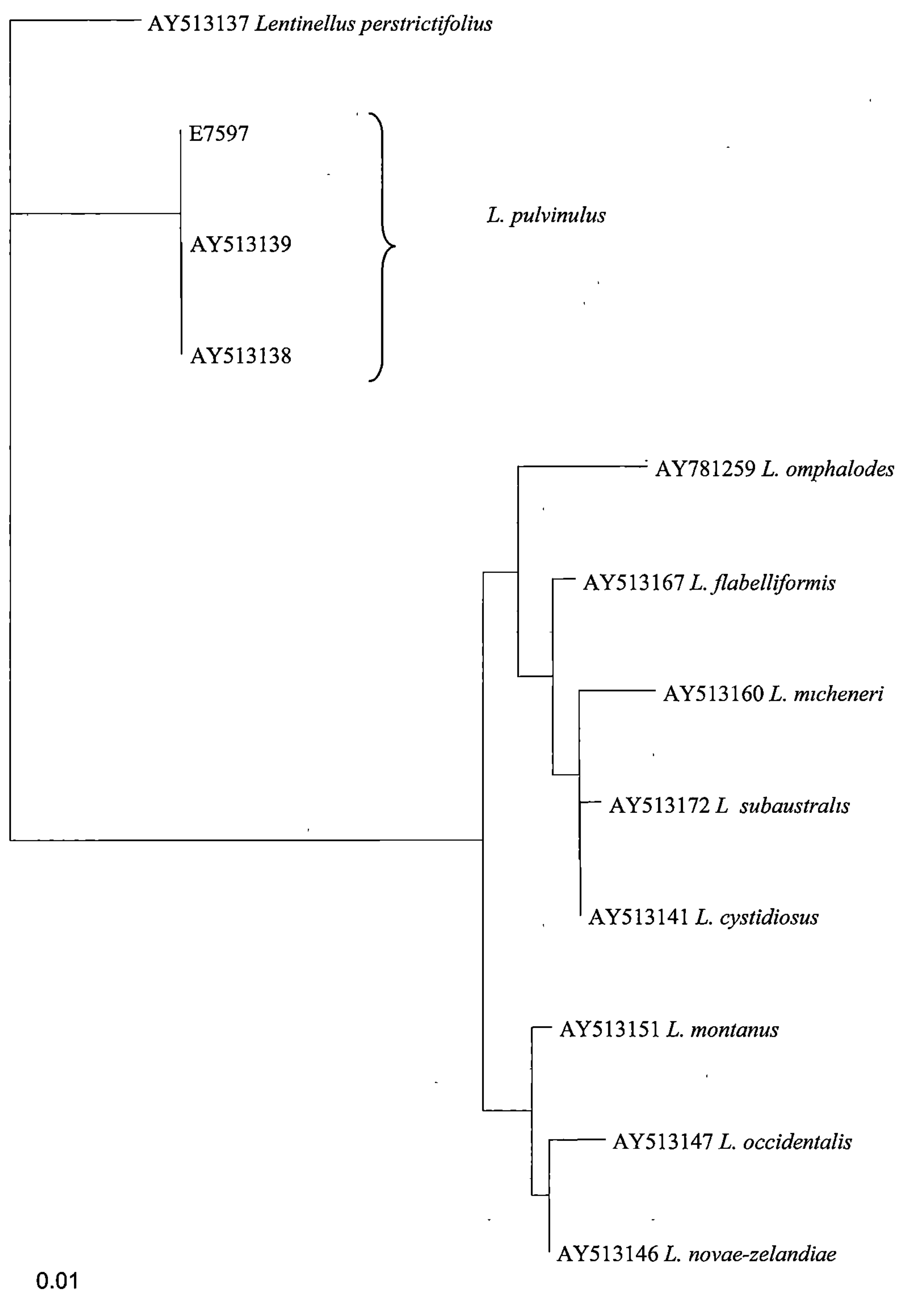


Figure A2.4.7: Maximum likelihood tree from analysis of ITS sequences of isolate E7597. The outgroup *Lentinellus perstrictifolius* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

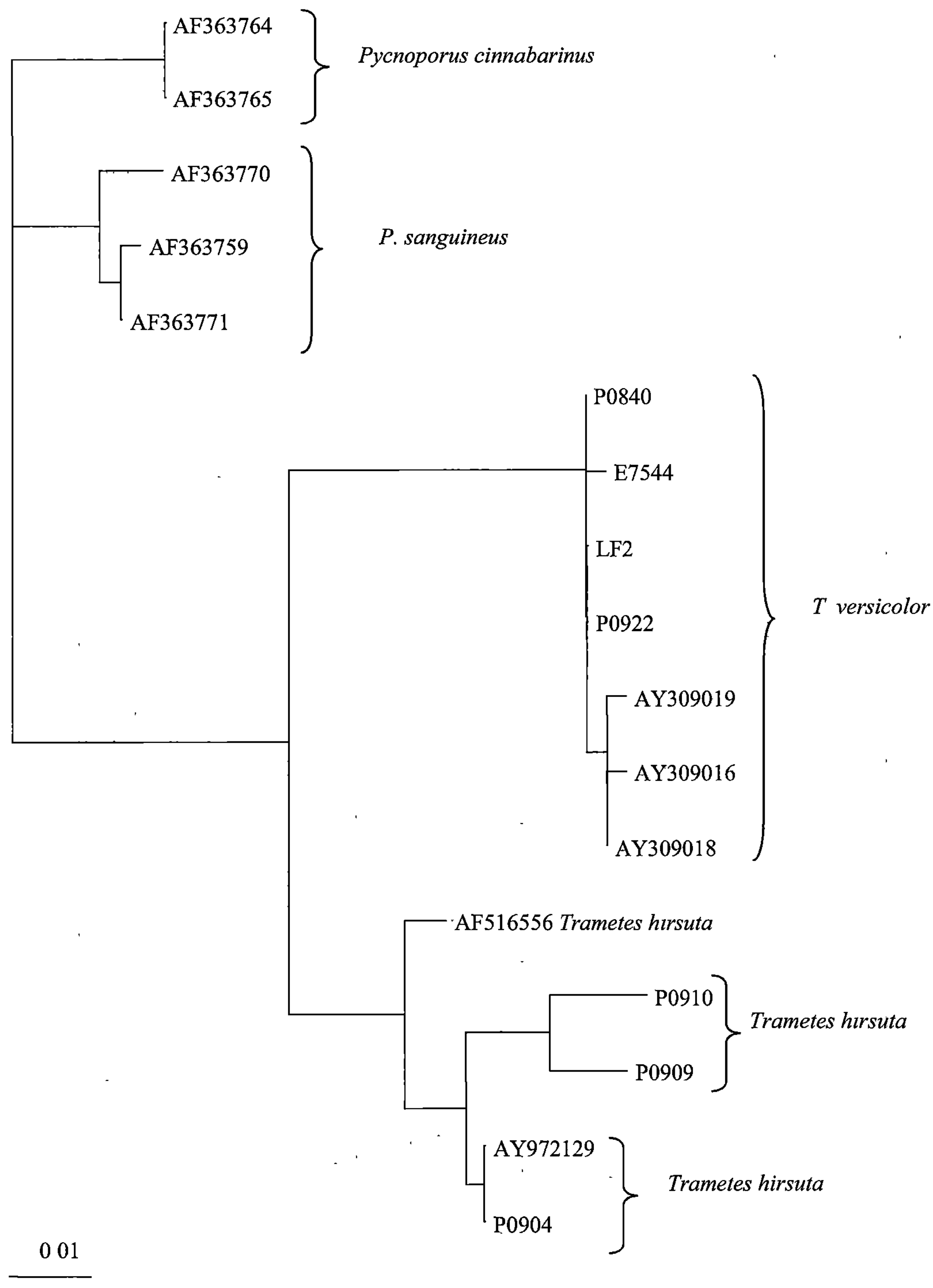


Figure A2.4.8: Maximum likelihood tree from analysis of ITS sequences of isolates P0904, P0909, P0910, P0922, P0840, LF2 and sporocarp E7544. The outgroups *Pycnoporus cinnabarinus* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

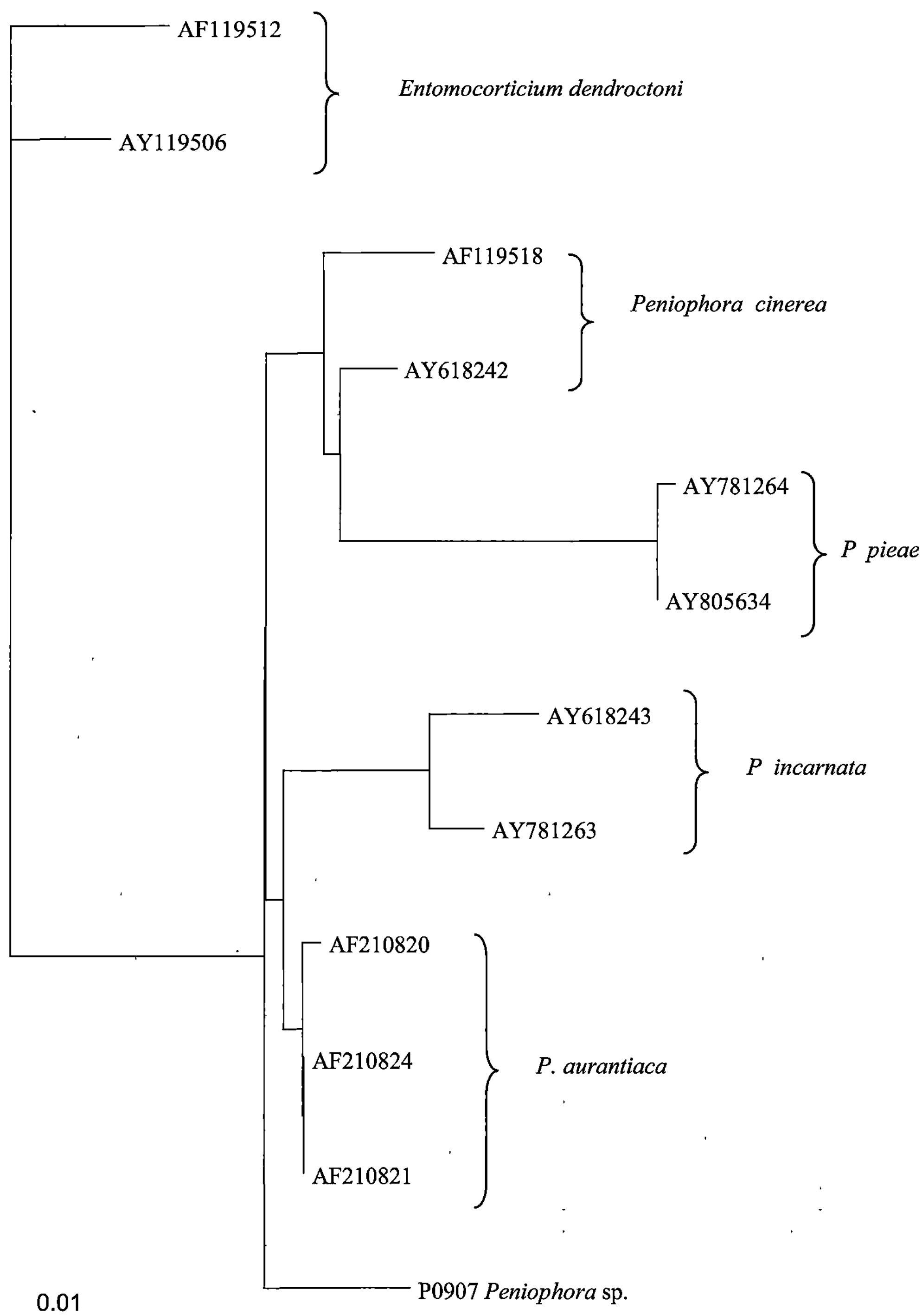


Figure A2.4.9: Maximum likelihood tree from analysis of ITS sequences of isolate P0907. The outgroup *Entomocorticium dendroctoni* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

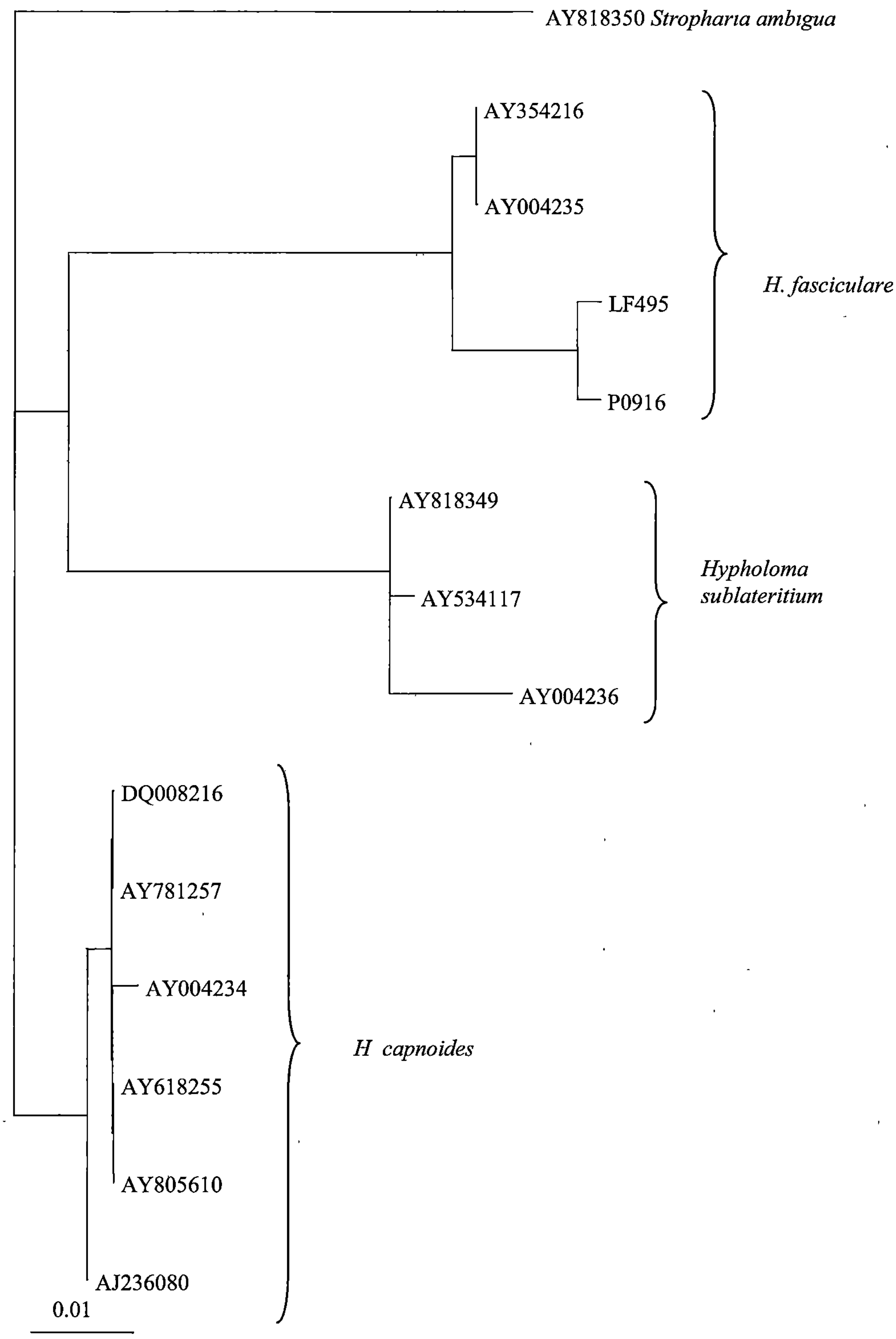


Figure A2.4.10: Maximum likelihood tree from analysis of ITS sequences of isolates P0916 and LF495. The outgroup *Stropharia ambigua* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

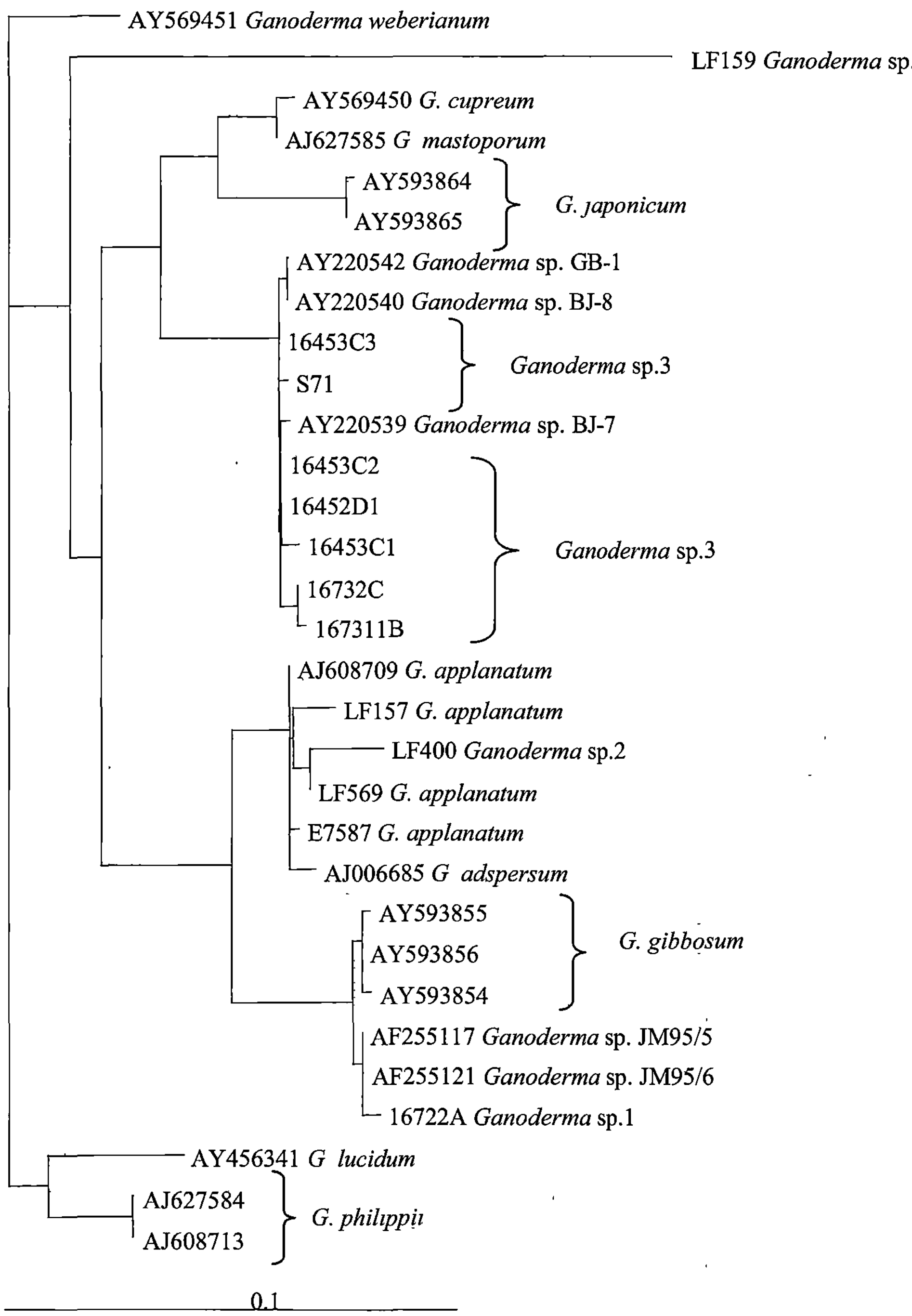


Figure A2.4.11: Maximum likelihood tree from analysis of ITS sequences of sporocarps E7587, 16722A, 167311B, 16732C, 16453C1, 16453C2, 16453C3, 16452D1, isolates LF157, LF159 LF400, LF569 and S71. The outgroup *Ganoderma weberianum* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 10%.

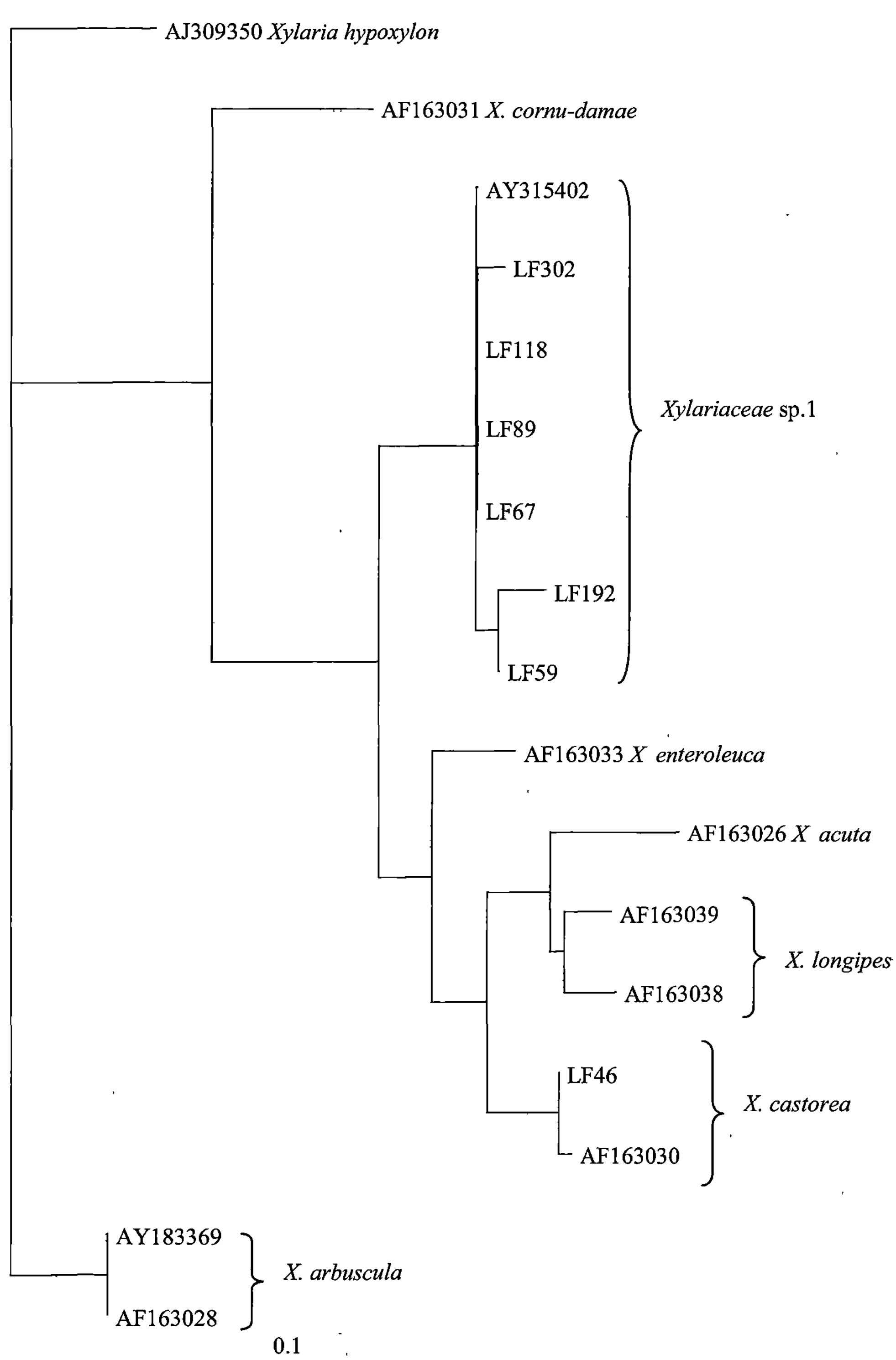


Figure A2.4.12: Maximum likelihood tree from analysis of ITS sequences of isolates LF46, LF59, LF67, LF89, LF118, LF192 and LF302. The outgroup *Xylaria hypoxylon* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 10%.

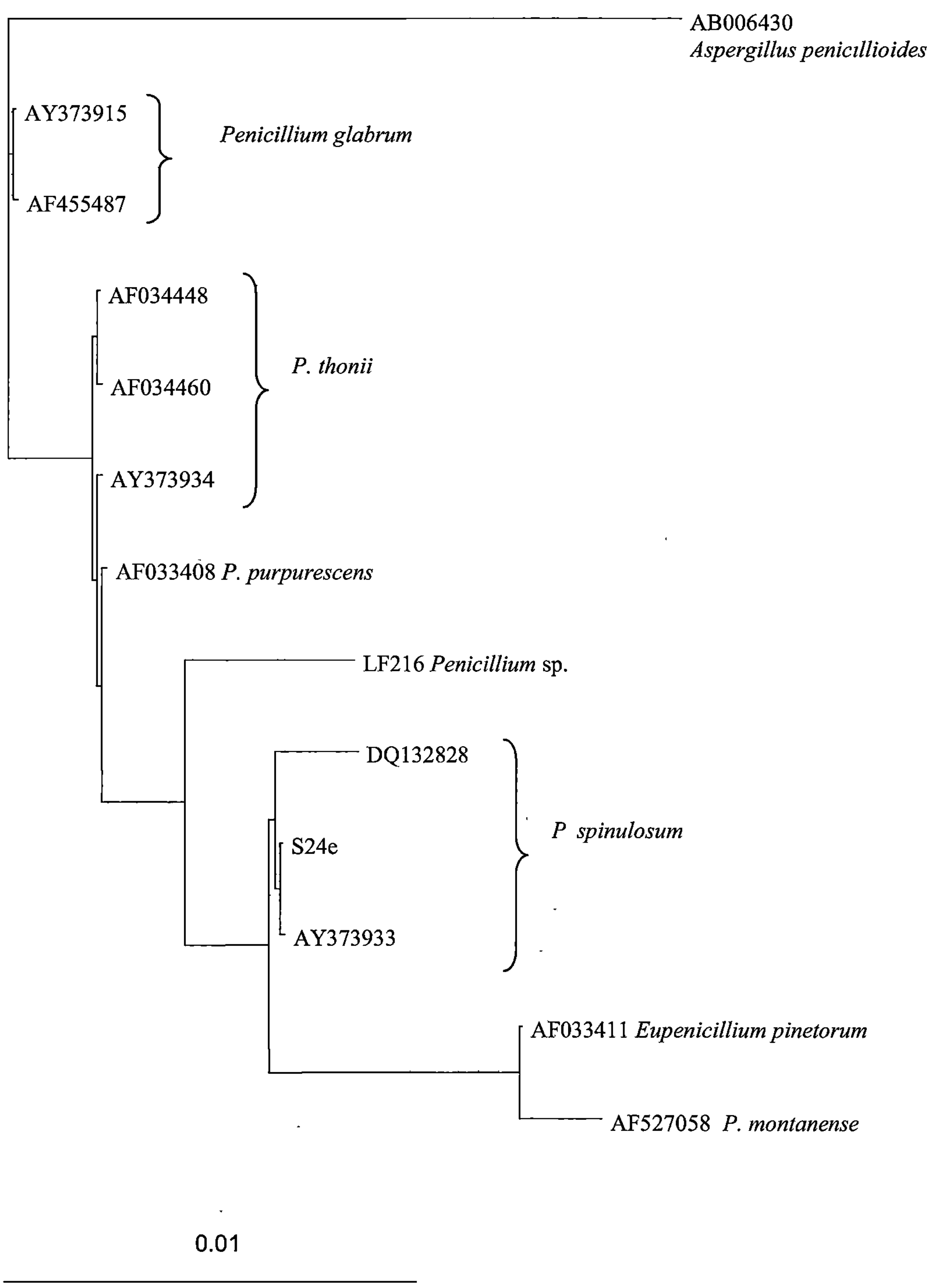


Figure A2.4.13: Maximum likelihood tree from analysis of ITS sequences of isolate LF216 and S24e (cloning sample). The outgroup *Aspergillus penicillioides* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

Note: *Eupenicillium pinetorum* = *Penicillium velutinum*

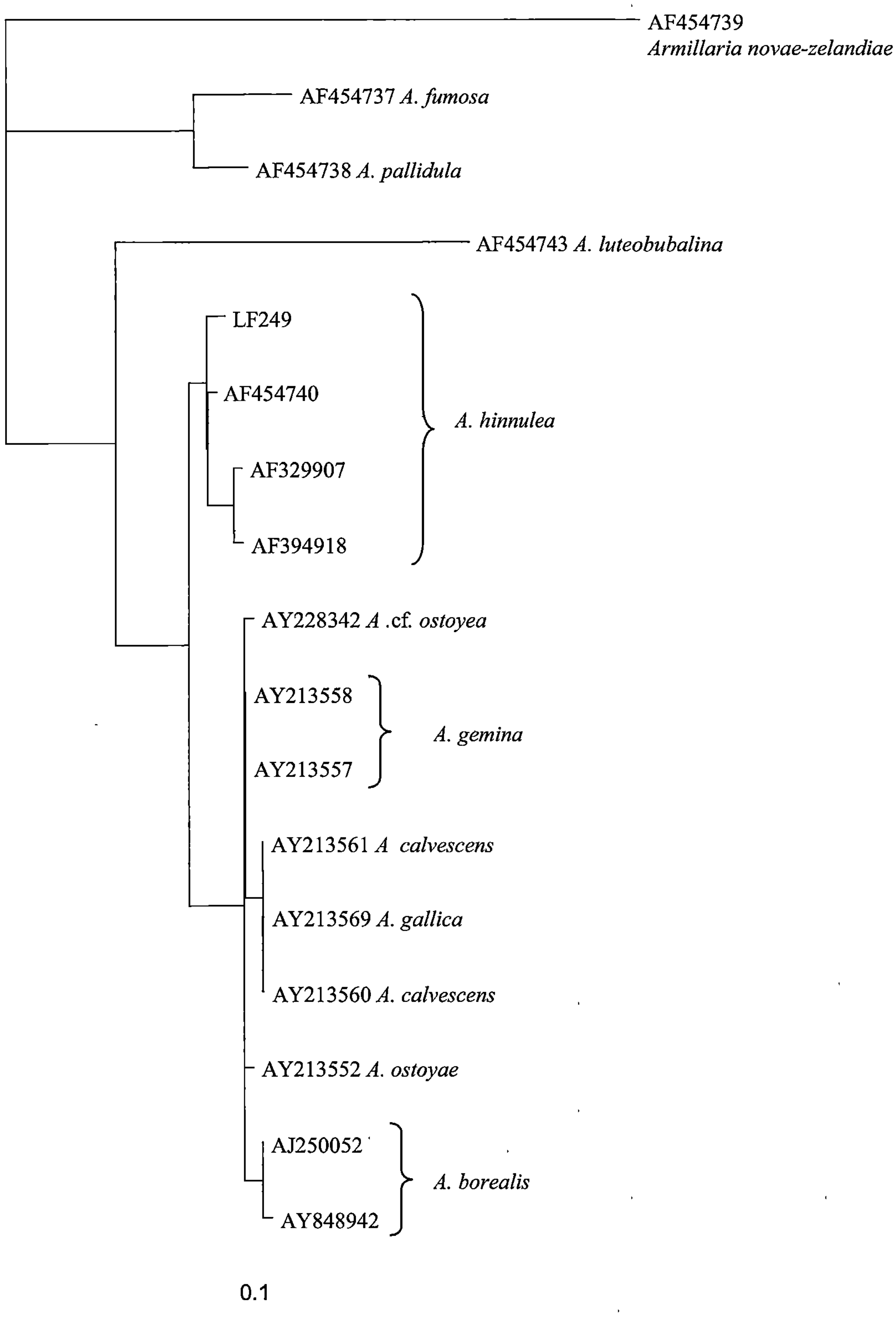


Figure A2.4.14: Maximum likelihood tree from analysis of ITS sequences of isolate LF249. The outgroup *Armillaria novae-zelandiae* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 10%.

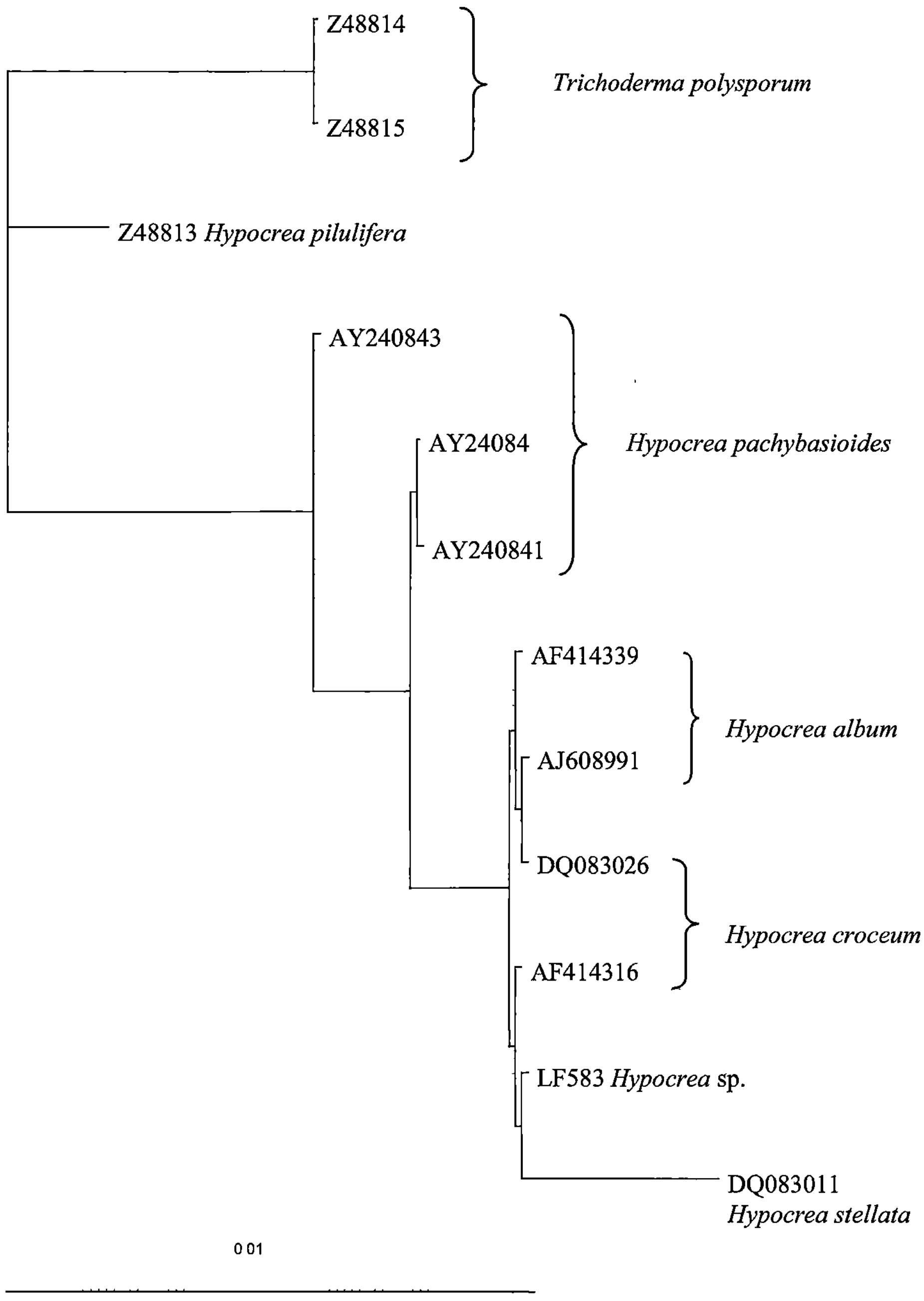


Figure A2.4.15: Maximum likelihood tree from analysis of ITS sequences of isolate LF583. The outgroups *Trichoderma polysporum* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

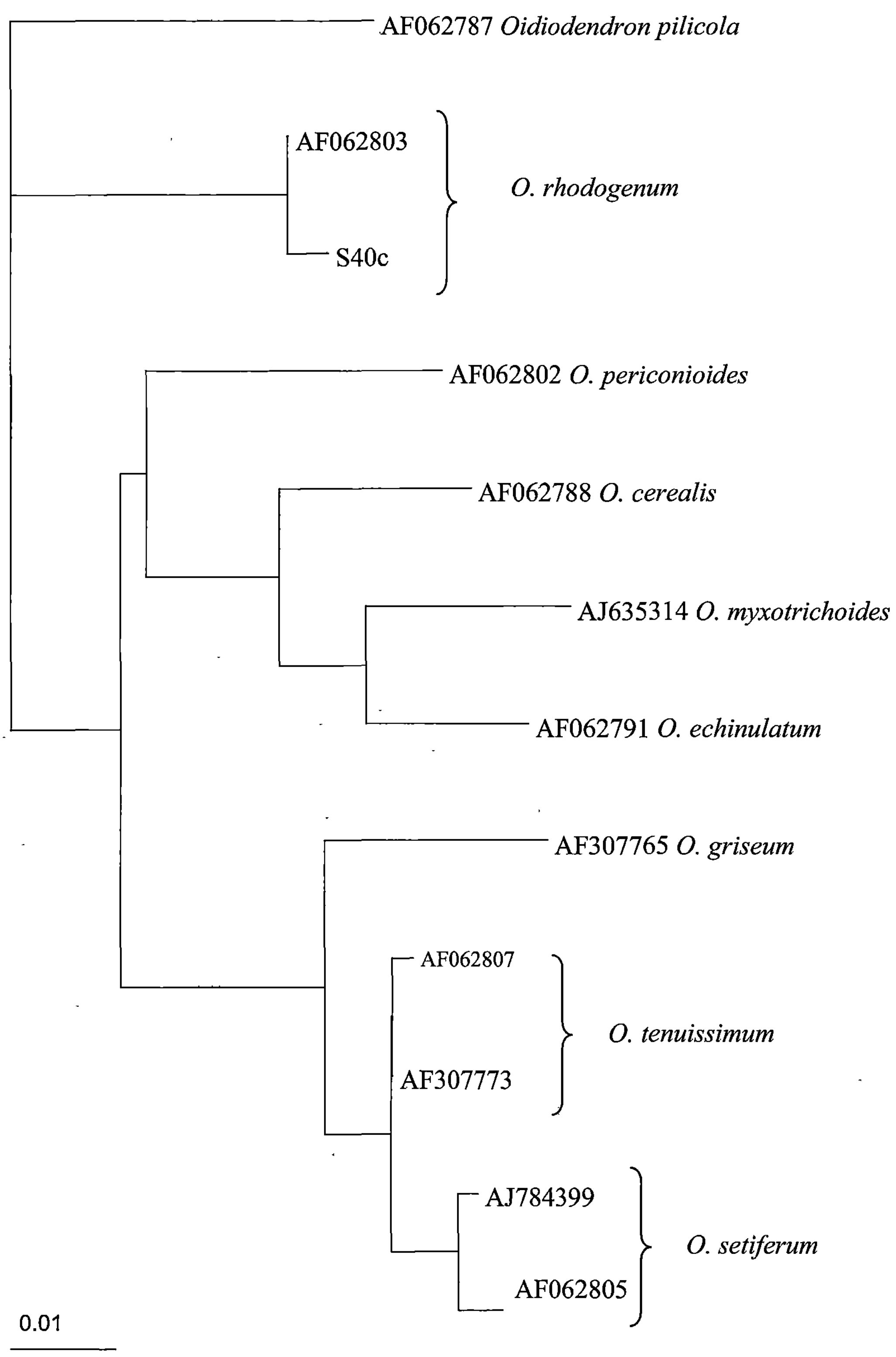


Figure A2.4.16: Maximum likelihood tree from analysis of ITS sequences of S40c (cloning sample). The outgroup *Oidiodendron pilicola* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

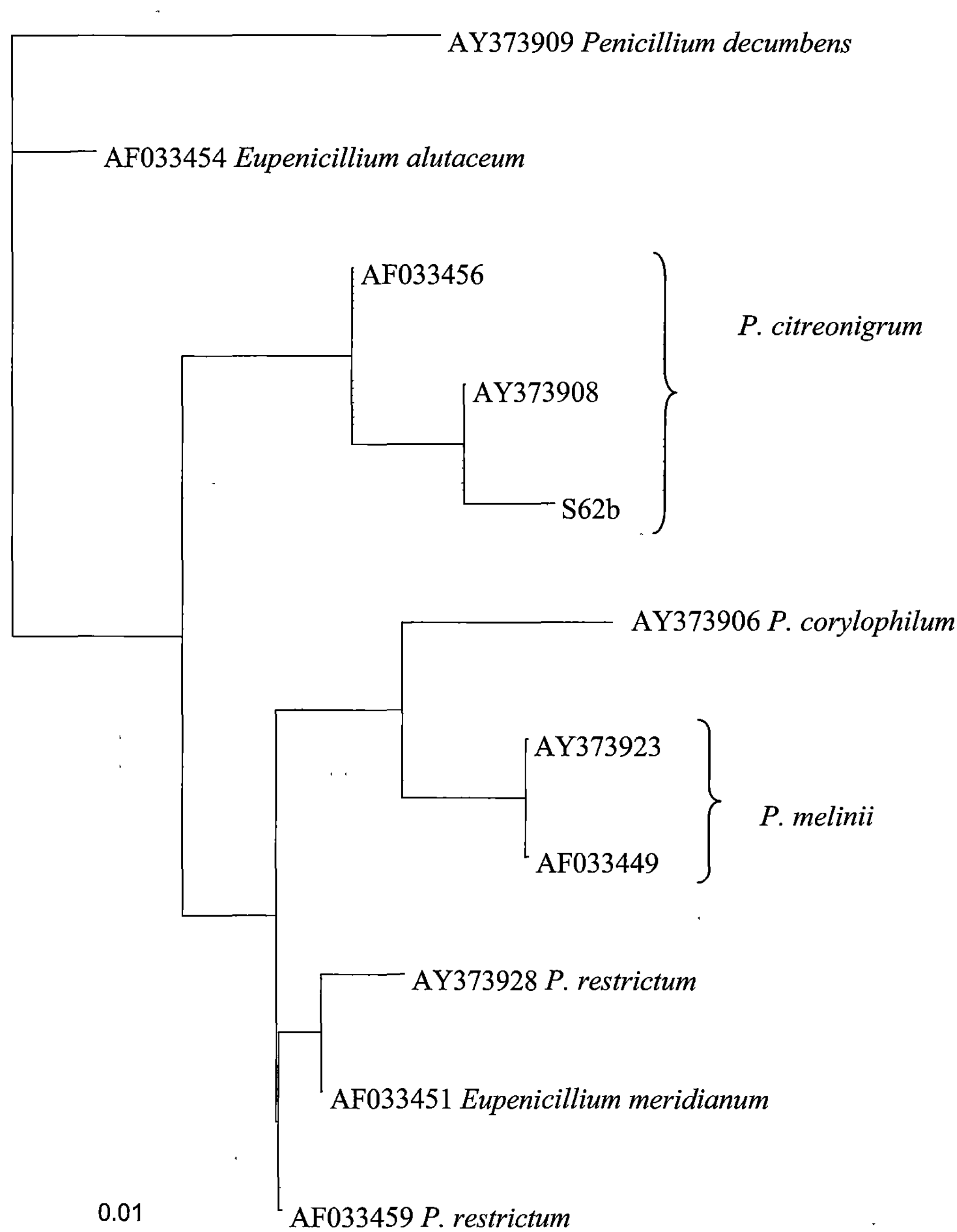


Figure A2.4.17: Maximum likelihood tree from analysis of ITS sequences S62b (cloning sample). The outgroup *Penicillium decumbens* and reference sequences are downloaded from GenBank (see above dendrogram for accession numbers). The bar represents an expected sequence variation of 1%.

Note: *Eupenicillium meridianum* = *Penicillium decumbens*